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THE STRUCTURAL BASIS OF THE EVOLUTION OF THE RESPIRATORY MECHANISM IN CHELONIA*

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THE origin of the chelonians is shrouded in mystery. Transitional ancestral forms are practically unknown. Nor is the evolution of the group any better known. The comparative study of chelonian features sheds little light on the subject, since they follow a monotonous pattern in the various families.

Since in these reptiles the ribs have been rendered immovable, the intercostal muscles have disappeared and the body wall turned rigid. The mechanism of respiration was a puzzle from the very early days. From Malpighi, Cuvier, and Agassiz to some recent investigators like Wolf (1933), Ludicke (1936), Noble and Noble (1940) and McCutcheon (1943), the subject remained a live issue. Wolf was the first to suggest that the movements of the limbs in and out of the shell respectively caused expiration and inspiration. Ludicke in observing the throat movements suggested that the aquatic species could swallow air, unlike the terrestrial forms like *Testudo gracea*. Cloacal respiration is also believed to be present in certain aquatic species.

McCutcheon (1943) performed some interesting experiments on turtles and as a result attributed to the flank cavity muscles and the muscular membranes which enclose the viscera, a role similar to that of the body wall in other reptiles, in bringing about pulmonary action, while he contended that the pressure created by the throat movements is associated with olfaction and not respiration.

Recently we (1954 and 1955 a) reported the occurrence of a pair of striated muscle sheaths probably homologous to the intercostal muscles, encasing the lungs in *Lissemys punctata*. When the muscle sheaths were made to contract by electrical stimulation of their nerves, expiration occurred. Conversely when the muscle sheaths relaxed, inspiration took

* A preliminary report was presented at the XVth International Congress of Zoology at London, 1958. *Proceedings*, (1959), 957.

place. Similar sheaths were found also in another chelonian *Geomyda, trijuga*, with the difference that in this turtle the sheaths were partly muscular and partly membraneous. This set of muscle sheaths is a new find and the bellows-like action of the muscle sheaths is a unique respiratory mechanism. These results stimulated us to make a more extensive study of the mechanism of respiration in the Chelonia.

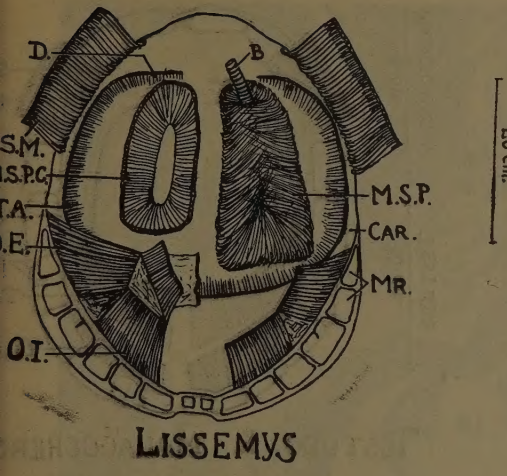
Material and Method

The following is a list of chelonians examined: *Lissemys punctata*, pond turtle; *Trionyx gangeticus*, river turtle; *Chelydra serpentina*, lake and river turtle; *Eretmochelys imbricata*, marine turtle; *Geomyda trijuga*, semi-fresh-water turtle; *Testudo elegans* and *Malcochersus tornieri*, both terrestrial forms. These included representatives from fresh-water, marine and terrestrial habitats and their study comprised muscles enclosing the viscera, flank cavity muscles, lung musculature and the structure of the lung. For studying the detailed structure of the lung, the tissue was fixed in Bouin's fluid and microtome sections of 5μ thick were cut. They were stained with haematoxylin and eosin.

Observations

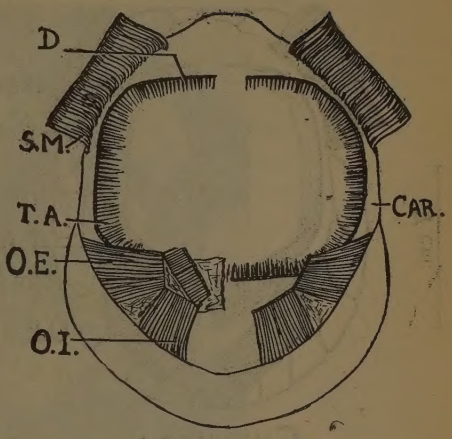
Flank cavity muscles: The flank cavity muscles consist of the *serratus magnus* anteriorly and the *obliquus obdominis* posteriorly. The *serratus magnus*, though a trunk muscle arising from the antero-lateral border of the carapace at its union with the plastron, is functionally a muscle of the shoulder girdle. The anterior half of the muscle is inserted on the postero-ventral border of the scapula, while the posterior half running medially gains insertion on the antero-dorsal border of the coracoid. The muscle acts as a depressor and lateral rotator of the shoulder effecting slight abduction of the arm. When such a movement is brought about, an increase in the size of the body cavity is brought about. This facilitates inspiration. The origin, course and insertion of this muscle are essentially similar in all the chelonians examined, though in the less active forms Geomyda, Testudo and the soft-shelled tortoise Malacochersus the muscle is less well developed.

The *obliquus abdominis* in Lissemys and Trionyx is made up of two parts, the *obliquus abdominis externus* and the *obliquus abdominis internus*. The former is a flat muscle which arises from the lateral border of the carapace at the level of the first and second marginals in Lissemys and from the corresponding region in Trionyx in which no marginals are



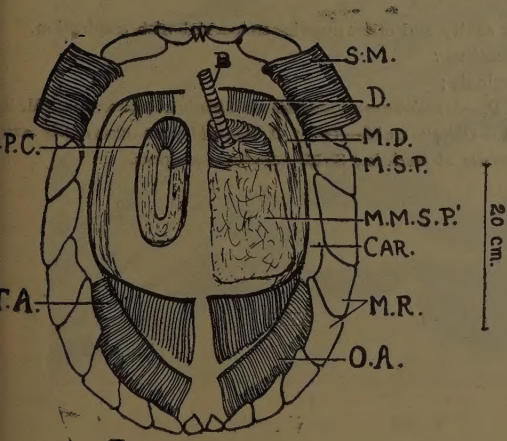
LISSEMYS

Fig. 1



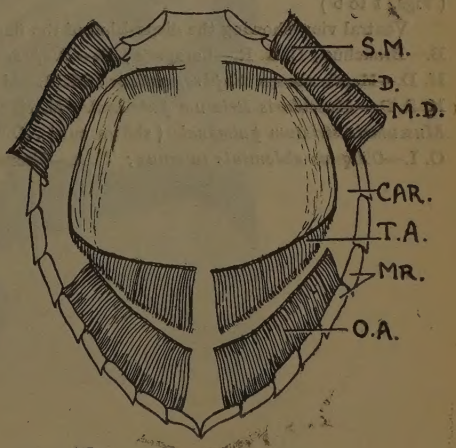
TRIONYX

Fig. 2



GEOMYDA

Fig. 3



ERETMOCHELYS

Fig. 4

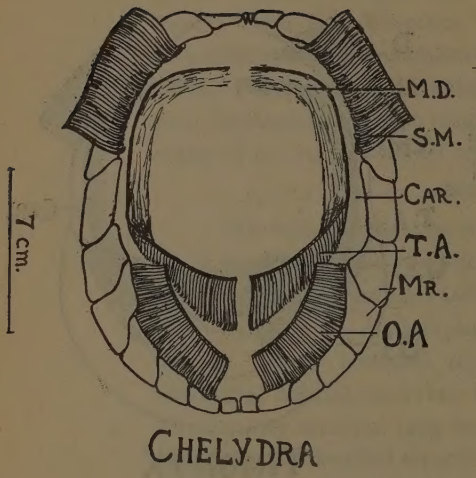


Fig. 5

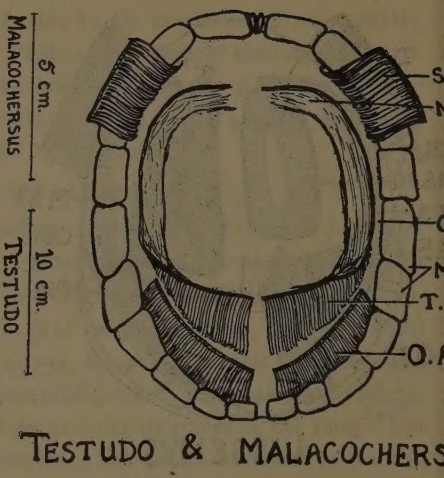


Fig. 6

(Figs. 1 to 6)

Ventral view showing the disposition of the flank cavity and other muscles concerned with respiration.

B.—Bronchus ; C. A. R.—Carapace ; D.—*Diaphragmaticus ;*
M. D.—Membraneous *diaphragmaticus ;* M. R.—Marginals ;
M. S. P.—*Muscularis striatum pulmonale ;* M. M. S. P.—*Membraneous muscularis striatum pulmonale* ; M. S.
Muscularis striatum pulmonale (shown cut) ; O. A.—*Obliquus abdominis* ; O. E.—*Obliquus abdominis exte*
O. I.—*Oblique abdominis internus ;* T. A.—*Transversus abdominis* ; S. M.—*Serratus magnus.*

Fig. 7

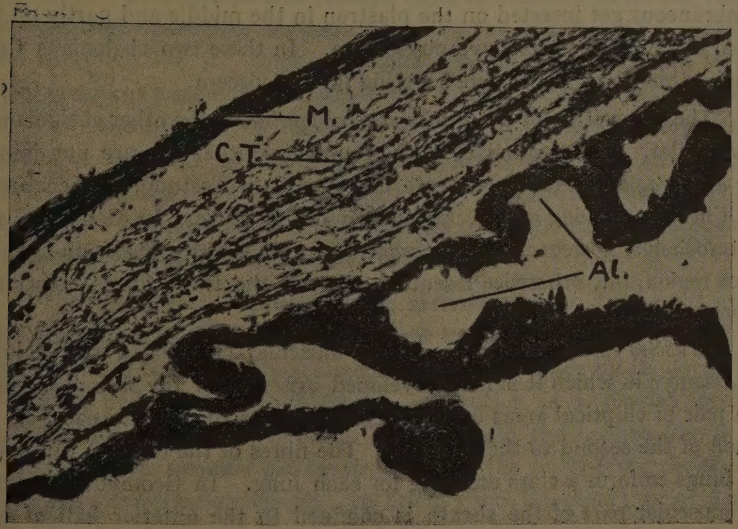


Fig. 8

Section of the lungs of Lissemys and Trionyx respectively.
Al.—Alveoli ; C. T.—Connective Tissue ; M.—Membrane ;
M. S. P.—*Muscularis striatum pulmonalis.*

differentiated. It runs obliquely, medially and posteriorly and then becomes tendinous. Thereafter it joins the membraneous part of the *transversus abdominis* to gain a common insertion on the dorsal aspect of the pubic symphysis. The *obliquus abdominis internus* arises from the lateral border of the costal plates corresponding to the third and fourth marginals. It runs obliquely, medially and anteriorly and after becoming tendinous joins the *obliquus abdominis externus* to gain a common insertion. These muscles when contracting bring about the movements of the pelvic girdles and also decrease the pressure in the body cavity by increasing its size. Along with the *serratus magnus* they facilitate inspiration. In the other chelonians the *obliquus abdominis* is a single large flat muscle, having similar associations.

Muscles enclosing viscera : These consist of the *diaphragmaticus* anteriorly and the *transversus abdominis* posteriorly. The disposition of these muscles is very similar in Lissemys and Trionyx. They arise from the inner lateral border of the carapace from the first to the seventh ribs in these chelonians, with the difference that in Trionyx there is a wider area of origin. They then proceed laterally downward and after becoming membraneous get inserted on the plastron in the middle and partly on the dorsal aspect of the pubic symphysis also. In these two chelonians these muscles thus form a complete covering for the viscera.

The disposition of the *transversus abdominis* in the other chelonians is very similar. The two halves of the muscle on either side are not joined. The *diaphragmaticus* is degenerate completely in Testudo, Malacochersus and Chelydra leaving only a membrane in its place. In Geomyda and Eretmochelys, however, the *diaphragmaticus* is slightly fleshy in the anterior region, but otherwise resembles that in the three forms mentioned above.

Muscles encasing the lungs : The muscular covering over the lungs in Lissemys in which it is best developed arises from the marginal tracts of a pair of elliptical areas on the ventral aspect of the carapace, in the region of the second to the fifth ribs. The fibres of the muscles run under the lungs to form a close covering for each lung. In Geomyda, however, the muscular part of the sheath is confined to the anterior half of each lung the posterior half being membraneous. This muscle is innervated by the intercostal nerves. We propose to call this muscle by the name *muscularis striatum pulmonale.* In the other chelonians examined the muscle is represented by a thin membrane formed of connective tissue.

Discussion

The present investigations throw some light on the evolution of the respiratory mechanism in the Chelonia. With the formation of the carapace and plastron and the ribs rendered immovable, the early chelonians must have utilized the internal intercostal muscles to pump air out of the lungs. The newly discovered muscle sheath around the lungs in Lissemys perhaps shows a primitive condition, and if so this muscle sheath is homologous with the internal intercostal muscles. Recent studies of Williams and McDowell (1952) on the plastron in Testudinata and Trionychidae have shown that the Lissemydinae are a primitive group and our studies (1955b, '55c, '57, '58) on chelonian myology also suggest the primitiveness of the subfamily.

In all the other chelonians studied except in Geomyda, the lung muscles are reduced to a mere membrane and the condition noted in Geomyda is transitional, with only the anterior portion of the lungs, enveloped by the muscles. With regard to the muscles enclosing the viscera, the *diaphragmaticus* and the *transversus abdominis*, variations have been noted. In Lissemys and Trionyx these two muscles cover the viscera. The *diaphragmaticus* has degenerated in all the others except Geomyda and Eretmochelys in which remnants are present. In place of the *diaphragmaticus* a membrane is present in those in which it has degenerated. The primitiveness of Lissemys and Trionyx and the intermediate position of Geomyda is supported by the study of chelonian skulls by Smith (1935) who has shown that the jugulo-quadrate bar characteristically present in Lissemys and Trionyx appears to be gradually disappearing in Geomyda and completely lost in Testudo.

The interesting conclusions to be arrived at from the study of the respiratory muscles, are not the variations in the various muscles, but the replacement of the older type of mechanism in Lissemys by the latter one in Testudo and others. In Lissemys, inspiration is a passive process, while expiration is the active one. This is due to the bellows-like action of the *muscularis striatum pulmonale*, aided by the outer-lying *diaphragmaticus* and *transversus abdominis*. In Trionyx too, the same type of inspiration takes place, though the inner layer of muscle sheaths *(muscularis striatum pulmonale)* has degenerated. The degeneration has resulted in yielding more space for the lungs to expand into, while the outer muscles alone being sufficient to bring about lower pressure around the lungs to

cause inspiration and sufficient external pressure around them on contraction to cause expiration.

With the degeneration of the *diaphragmaticus* in all the forms other than Lissemys, Trionyx and Eretmochelys, expiration is no longer an active process. In these the flank cavity muscles by their action bring about inspiration and when they relax, expiration passively takes place. Geomyda while breathing like Testudo tells its own tale of being a half-way house in respect of the degeneration of the *muscularis striatum pulmonale* and *diaphragmaticus*.

Summary

1. Respiratory mechanism in Lissemys appears to be the most primitive with a layer of muscle around each lung and also an outer layer of muscles around the viscera. These muscles by their contraction bring about expiration and by relaxation inspiration. The same type of mechanism works in Trionyx, though the muscle sheaths around the lungs have degenerated.

2. In others the flank cavity muscles replace the muscles round the lungs and around the viscera as respiratory muscles. The flank cavity muscles by their contraction cause inspiration and by their relaxation expiration.

3. Remnants of the lung muscle *muscularis striatum pulmonale* as well as of the *diaphragmaticus* are still retained in Geomyda. Those of diaphragmaticus are left in Eretmochelys.

Acknowledgement

We are grateful to Dr. E. Williams of Harvard university, U. S. A. for very kindly sending us two well preserved specimens of *Malacochersus tornieri*.

References

Agassiz, J. L. R. 1857 Contribution to the Natural History of the United States, **1** : 281.

George, J. C. and Shah, R. V. 1954 The occurrence of a striated outer muscular sheath in the lungs of *Lissemys punctata granosa* Schoepff. *J. Anim Morph. Physiol.* **1**. 1. 13.

George, J. C. and Shah, R. V. 1955 a Respiratory mechanism in the Chelonia. *Ibid.* **1**. 2. 30.

George, J. C. and Shah, R. V.	1955 b	The myology of the head and neck of the Indian tortoise, *Testudo elegans*. *Ibid*, 2, 1, 1.
George, J. C. and Shah, R. V.	1955 c	The myology of the chelonian trunk and tail. *Ibid*, 2, 49.
George, J. C. and Shah, R. V.	1957	The myology of the chelonian limb 1. The forelimb of *Lissemys punctata* *Ibid*, 4, 81.
George, J. C. and Shah, R. V.	1958	The myology of the chelonian limb 2. The hind limb musculature of *Lissemys punctata*. *Ibid*, 5, 21.
Ludicke, M.	1936	Uber die Atmung von *Emys orbicularis*. *L. Zoo. Jahrb. Abt. allg. Zool. u. Physiology*. 56 : 83-106.
Malcom, A. Smith	1931	The Fauna of British India.
McCutcheon, F. H.	1943	The respiratory mechanism in turtles. *Physiol. Zool.* 16. 3.
Noble, G. A. and Noble, E.R.	1940	Anatomy of Turtle. Stanford University Press.
Williams, E. E. and McCowell, S. B.	1952	The plastron of the soft-shelled turtles (Testudinata, Trionychidae). A new interpretation. *J. Morph.* 90.
Wolf, S.	1933	Zur Kenntnis von Bau and Funktion der Reptilienlunge. *Zool. Jahrb. Abt. Ant. u. Ont.* 57 : 139-90.

A STUDY OF THE CEPHALO-PHARYNGEAL SKELETON OF THE LARVA OF *MUSCA DOMESTICA NEBULO* FABR.

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NEWSTEAD (1908) has studied the habits and life history of *Musca domestica*, L. Howard (1911) has described the bionomics and control of *Musca domestica*, L. Hewitt (1914) was the first to work out in detail the bionomics, morphology, disease relationships and control of the same fly. Keilin (1915) has made a valuable contribution to our knowledge of the dipterous larvae. Tao (1927) made a very careful study of the early stages of several muscoid flies. West (1951) has dealt with the natural history, medical importance and control of *Musca domestica L.* Deoras and Ranade (1957) have described the life history of the commonest Indian housefly, *Musca domestica nebulo*, Fabr. However, the cephalo-pharyngeal skeleton has not been described in detail. The cephalo-pharyngeal skeleton is useful to determine the exact instar of the larva. Taking into consideration this importance, this study of the structure of the cephalo-pharyngeal skeleton was undertaken by the author.

Material and Methods

The flies were bred in large numbers in the laboratory, by feeding the larvae on a semisolid mixture of wheat bran, yeast and water, and the adults on milk and sugar. The larva were killed in hot water and kept in 5% KOH overnight. The cephalo-pharyngeal skeleton was dissected and after the usual processes of dehydration was mounted in canada balsam. The drawings were made with camera lucida.

Observations

The eggs hatch within 24 hours after oviposition. At 24°-35°C hatching takes place within 8-12 hours.

The larva or maggot passes through three stages or instars which means that in the course of development, it moults twice with distinctive changes of structure at each ecdysis. Immediately after emergence the larva wriggles actively inside the breeding media. By forward and sideway thrusts with the head assisted by the cephalo-pharyngeal skeleton, it

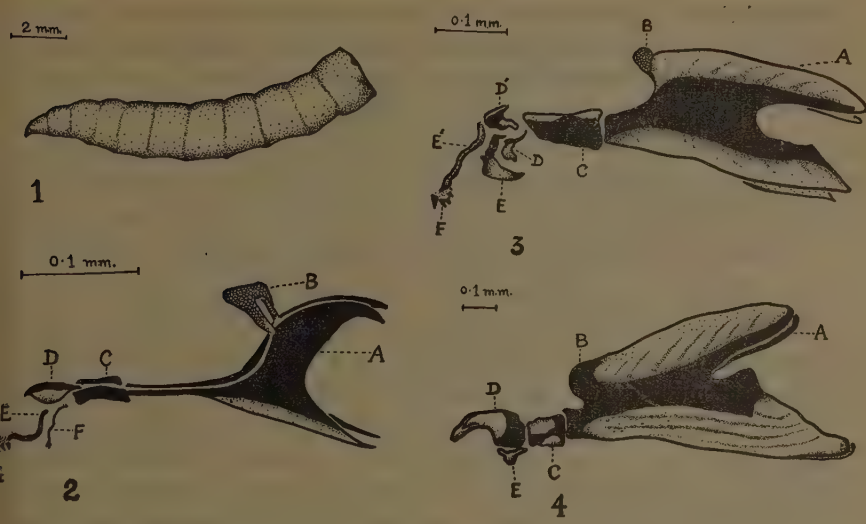

Fig. 1—Third instar larva.

Fig. 2—Cephalo-pharyngeal skeleton of the first instar larva.
(A) Lateral pharyngeal sclerites; (B) Dorsal sclerites; (C) Hypostomal sclerites; (D) Median hook; (E) Dorsal elongated sclerite; (F) Ventral elongated sclerite; (G) Supra-buccal denticles.

Fig. 3—Cephalo-pharyngeal skeleton of the second instar larva.
(A, B, C) same as in Fig. 2.; (D) Posterior pair of ventral sclerites (D') Posterior pair of dorsal sclerites; (E) Anterior pair of ventral sclerites; (E') Anterior pair of dorsal sclerites; (F) Supra-buccal denticles.

Fig. 4—Cephalo-pharyngeal skeleton of the third instar larva.
(A, B, C) Same as in Fig. 2.; (D) Mandibular sclerite; (E) Dentate sclerite.

makes a tunnel and goes deeper and deeper in the food. It is negatively phototropic. The first instar larva is 1.3 mm. long. It moults within 20 hours after hatching. The second instar larva is 3 mm. long and moults within 24 hours. The third instar larva is 10 mm. long and feeds for 6-10 days before pupating.

The larval instars have twelve easily recognisable segments, the 2nd segment being usually double. A total of 13 segments can be counted. They have no eyes, legs, antennae or other appendages. The mouth parts can be said to consist only of the cephalo-pharyngeal skeleton which is lodged in the oral lobes. Segments 2–5 have complete spinose rings and segments 6-12 have on their ventral side crescent-shaped spiniferous pads

which help the larvae in locomotion. The last, the 13th or anal segment has two lobes which are also useful in locomotion. The anterior end of the larva is tapering and the posterior end is broad.

Cephalo-pharyngeal skeleton of the first instar larva: (Fig. 2). It is slender, chitinous and consists of (1) a pair of lateral pharyngeal sclerites (A), (2) a dorsal sclerite (B), (3) a pair of hypostomal sclerites (C), (4) a median hook (D), (5) one dorsal (E) and one ventral (F) pair of elongated sclerites, and (6) supra-buccal denticles (G).

The lateral pharyngeal sclerites (A) are the largest; they are deeply incised posteriorly thus forming the dorsal and ventral processes. These processes are connected antero-dorsally by a curved dorsal sclerite (B). It is less chitinous and has a network structure, which serves to differentiate the family Muscidae from the Calliphoridae. The lateral pharyngeal sclerities are united ventrally. Anteriorly they are produced to form a pair of slender processes to which are articulated, a pair of strong., rectangular, chitinous hypostomal sclerites (C). Anterior to these hypostomal sclerites is the median hook (D). On either side of this hook lie two pairs of slender, enlongated and curved sclerites. The dorsal pair (E) is longer and articulated anteriorly with the supra-buccal denticles (G). The ventral pair (F) is smaller and shorter and articulates posteriorly with the hypostomal sclerite (C).

Cephalo-pharyngeal skeleton of the second instar larva: (Fig. 3). It is more chitinized than that of the first instar larva and consists of:— (1) a pair of lateral pharyngeal sclerites (A), (2) a dorsal sclerite (B), (3) a pair of hypostomal sclerites (C), (4) two pairs of ventral sclerites (D, E), and (5) two pairs of dorsal sclerites (D', E') and (6) supra-buccal denticles (F).

The lateral pharyngeal sclerites (A) are the largest. Their posterior sinuouses are curved in forming dorsal and ventral processes, which are thicker. These sclerites and are connected antero-dorsally by a curved dorsal sclerite (B). It is less chitinous and shows a net work structure. The anterior edges of the lateral pharyngeal sclerites are produced a little ventrally, and to them is articulated a pair of strong, chitinous, quadrilateral hypostomal sclerites (C). The posterior margins of these sclerites are broader than the anterior ones. Anterior to the hypostomal sclerites are situated four pairs of irregular sclerites, of which two pairs are dorsal and two ventral. Out of the two ventral sclerites the posterior pair (D)

consists of slender, small sclerites which articulate with the antero-ventral margin of the hypostomal sclerite (C). The anterior pair (E) consists of plough-shaped, strong sclerites which are articulated with the dorsally-situated pair of posterior sclerites. Out of the two dorsal pairs of sclerites, the posterior pair (D') consists of small, wing-shaped sclerites. The anterior pair (E') has elongated, straight chitinous sclerites, which articulate anteriorly with the supra-buccal denticles (F). In advanced second instar larva dorsal to the anterior pair of dorsal sclerites is present a pair of chitinized, hook-like mandibular sclerites.

Cephalo-pharyngeal skeleton of the third instar larva: (fig. 4) It is greatly chitinized, massive and consists of the following sclerites. (1) a pair of lateral pharyngeal sclerites (A), (2) a dorsal sclerite (B), (3) a pair of hypostomal sclerites (C), (4) a pair of mandibular sclerites (D) and (5) a pair of dentate sclerites. (E).

The lateral pharyngeal sclerites (A) are heavily chitinized plates, posteriorly wider and deeply incised. They are united ventrally forming a trough in which lodges the pharynx. Antero-dorsally these sclerites are united by the dorsal sclerite (B) which firmly fuses with them. The anterior edges of the lateral pharyngeal sclerites are produced ventrally a little distance and then they join with the hypostomal sclerites (C). These sclerites consist of two irregularly-shaped sclerites which are united ventrally. Anteriorly these are articulated to a pair of mandibular sclerites (D) which lie between the oral lobes. The mandibular sclerites are hook-like and the left sclerite is always shorter than the right. The basal extremities of these sclerites are broad and at each side there is a notch where the dentate sclerite (E) articulates.

Discussion

It will be found from the above description that the cephalo-pharyngeal skeleton of the muscid larvae is modified as the larva grows and moults. In Nematocerous Diptera (*e.g.* Mosquito larva) typical biting and chewing mouth parts are present. Among the Brachycera, the same parts, are somewhat variously modified, and the mandibles work in the vertical plane. In Cyclorrhapha to which the family Muscidae belongs, the typical mouth parts have undergone atrophy in correlation with the reduction of the head. Keilin (1955) has shown that in saprophagous larvae the floor of the pharyngeal sclerite is beset with longitudinal ridges which project into the cavity of the pharynx. In the case of larva feeding on

living animal or vegetable tissues, these ridges are found to be reduced or absent. In phytophagous larvae, the mandibular sclerites are toothed and in carnivorous larvae they are sharp and pointed, while in parasitic larvae the buccal armature is greatly reduced.

The changes which have led to the atrophy of the normal biting mouth parts in the larvae of Cyclorrhapha are closely associated with the backward shifting of the brain and the other structures of the region as a result of which the development of imaginal head takes place within the larval metathorax.

Deoras and Ranade (1957) have listed the works of several authors on the food requirements of housefly larvae. The actual food of the larva is not however, known, but it is believed that it absorbs only liquid food. The lateral pharyngeal sclerites have longitudinal ridges and the cephalo-pharyngeal skeleton as a whole is adapted for suction. The mouth parts vary in the succeeding instars and during ecdysis, along with the lining of the stomodaeum, proctodaeum, and the spiracles, the mouth parts are also shed. The larvae feed continuously burrowing constantly in the food medium. The mouth parts are useful in locomotion. They leave the food medium when it gets dried up or when the feeding is complete at the end of the third instar when they reach maturity. They then move out and search for a dry place for pupation.

Further work on the larval food and larval anatomy is in progress and the details will be published sometime later.

Summary

The cephalo-pharyngeal skeleton of the first, second and third instar larvae of the housefly is described in detail. Its structure varies in each instar. In first instar larva it is less chitinous and consists of several sclerites. In second instar larva, it undergoes several modifications with an increase in the number of sclerites. In third instar larva it is a massive, chitinous skeleton with less number of sclerites. Thus it helps to determine the exact instar of the larva.

Acknowledgements

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References

Deoras, P. J. and Ranade, D. R. 1957 Life history of the common Indian housefly, *Musca Domestica nebulo*, Fabr. *Ind. J. Ent.* 19, 15.

Hewitt, C. G. 1914 The Housefly, *Musca domestica*, L. Its structure, habits, development, relation to diseases and control. Cambridge, England.

Howard, L. O. 1911 The Housefly, Disease carrier, an account of its dangerous activities and of the means of destroying it. New York.

Keilin, D. 1915 Recherches sur les larves de Dipteres, Cyclorhaphes. *Bull. sci. Fr. et. Belg.* 49, 15.

Newstead, R. 1908 On the habits, life history and breeding places of the common housefly. *Ann. Trop. Med. Parasit.* 1, 507.

Tao, S. M. 1927 A comparative study of the early larval stages of some common flies. *Am. J. Hyg.* 7, 735.

West, L. S. 1951 The Housefly—its natural history, medical importance and control. New York.

STUDIES ON THE STRUCTURE AND PHYSIOLOGY OF THE FLIGHT MUSCLES OF BIRDS

5. Some Histological and Cytochemical Observations on the Structure of the Pectoralis*

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(2 Plates)

George and Jyoti (1955a) and George and Naik (1957a) recorded some observations on the structure of the breast muscle in a few birds and suggested the possibility of some relationship between the structure of the muscle and the mode of flight. Following this line of investigation a comparative study of certain structural peculiarities of the pectoralis in relation to function in some birds is presented here.

Material and Methods

The following birds used in the present study were either shot with air rifle or trapped alive.

A. Birds exhibiting shooting type of flight : Blue-tailed Bee-eater *(Merops superciliosus)*, House Sparrow *(Passer domesticus)*, Indian Robin *(Saxicoloides fulicata)*, Mahratta Woodpecker *(Liopicus maharattensis)*, Red-vented Bulbul (Molpastes hoemorrhous), White-breasted Kingfisher *(Halcyon smyrnensis)*.

B. Birds exhibiting flapping type of flight : Blue Jay *(Corasias bengalensis)*, Cattle Egret *(Bubulcus ibis)*, Common Babbler *(Argya caudata)*, Common House Crow *(Corvus splendens)*, Common Myna *(Acridotheres tristis)*, Crow Pheasant (*Centropus sinensis)*, Green Parakeet *(Psittacula Krameri)*, Koel *(Eudynamis scolopaceus)*, Paddy-Bird *(Ardeola grayi)*, Pied Crested Cuckoo *(Clamator jacobinus)*, Red-rumped Swallow *(Hirundo daurica)*, Red-wattled Lapwing *(Sarcogrammus indicus)*.

C. Birds exhibiting soaring flight : Common Pariah Kite *(Milvus migrans)*, Shikra *(Astur badius)*, White-backed vulture *(Pseudogype bengalensis)*.

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* The term pectoralis refers to the *pectoralis major* and does not include *pectoralis minor* which should be better named as *supracoracoideus*.

D. Nonflying birds: Domestic Fowl *(Gallus domesticus)*, Grey Partridge *(Francolinus pondicerianus)*.

General observations on the structure of the muscle were made from fresh frozen sections as well as frozen sections of muscle pieces fixed in 10% neutralised formalin.

Studies on the distribution of fat, glycogen and mitochondria in the pectoralis were confined only to the dove, parakeet, kingfisher, kite and fowl which were procured alive. The animals were decapitated and small pieces of muscle were cut out and fixed in appropriate fixatives. For the demonstration of fat, the muscle pieces fixed in Formol-Calcium were post-chromated (Baker, 1946), embedded in gelatin (Clark, 1947) and the sections were stained with Sudan Black B. For the demonstration of glycogen thin strips of muscle fixed in Rossman's fluid (at–10°C) were embedded in paraffin and sections were stained with Best Carmine. Some sections were also stained by PAS technique as described by Pearse (1954). The control sections were incubated in saliva. Prior to staining all sections, control as well as sample, were coated with celloidin. Altmann's method was adopted for the demonstration of mitochondria.

Observations

Histology: Group A and B: Among the birds studied, the bee-eater and the swallow are peculiar in so far as their pectoralis is composed uniformly of red fibres only. In others however, though the muscle is mainly made up of red fibres, an admixture of lighter coloured fibres is met with. Some of these light coloured fibres may be almost whitish. In the dove for example which is allied to the pigeon, the lighter fibres may be described as white. In the cattle egret and the kingfisher the lighter fibres are distributed throughout the muscle, though in the superficial layers they are more in number. In others on the contrary, lighter fibres are confined only to the superficial layers.

Group C: In the kite and vulture, the pectoralis can be divided into superficial and deeper parts according to the nature of the muscle fibres. The superficial part which forms the main bulk of the muscle contains only red fibres, whereas the deeper layer contains only light fibres, but the diameter of the fibres in the two regions does not show any significant difference. The separation between these two parts is complete in the vulture but incomplete in the kite. The pectoralis of the hawk

differs from that of the kite and vulture in that it is of mixed type, with light and red fibres distributed throughout the substance of the muscle.

Group D : In the fowl and partridge, all the muscle fibres are light in colour even though there is a great variation in the diameter of the fibres. Out of two specimens of partridge dissected, in one in the deeper layer of the pectoralis a small patch towards the anterior region showed a tinge of red, the microscopic examination of which showed the presence of light as well as red fibres.

Cytology : The colour of the muscle fibres is mainly due to the presence of the respiratory pigment myoglobin and granular inclusions (mitochondria and fat globules). When stained with Sudan Black B, the fat globules appear dark whereas the mitochondria are stained lightly. The red fibres in the pectoralis of birds contain heavy granular inclusions arranged in longitudinal streaks in between (Kolliker's) muscle columns (fig. 2), thus rendering the latter very conspicuous (figs. 1, 3 and 5) and the fibres in the pectoralis of the kite are no exception to this (figs. 1 and 2). This contradicts the earlier observations by George and Jyoti (1955a) and George and Naik (1957a) that the fibres in the pectoralis of kite are devoid of heavy granular inclusions. This was due to the fact that their observations on the granular inclusions were confined to the teased out muscle fibres, stained with Aceto-Carbol Sudan III. The latter does not reveal all the fat and moreover, large amounts of interstitial connective tissue and fat in the pectoralis of kite, renders the observation of sarcoplasmic inclusions in teased out preparations practically impossible. The muscle columns are less conspicuous in the light fibres of the mixed type of pectoralis. Due to decrease in the granular deposition, the treatment with Sudan Black B leaves the fibres of the fowl (fig. 4) and partridge and the white fibres of the dove (fig. 5), practically unstained.

Observations on the sections stained with Sudan Black B and the preparations made by Altmann's method show that the red fibres of the pectoralis of birds contain large number of mitochondria, while they are comparatively less in the light fibres. In the fibres of the fowl and partridge pectoralis and the white fibres in the pectoralis of dove, mitochondria are hardly detectable under the microscope. Paul and Sperling (1952) in the cyclophorase preparation of the fowl breast muscle observed few or no mitochondria. The electron microscope photographs of this muscle by Bennett and porter (1953) however, show some 'sarcosomes' quite clearly.

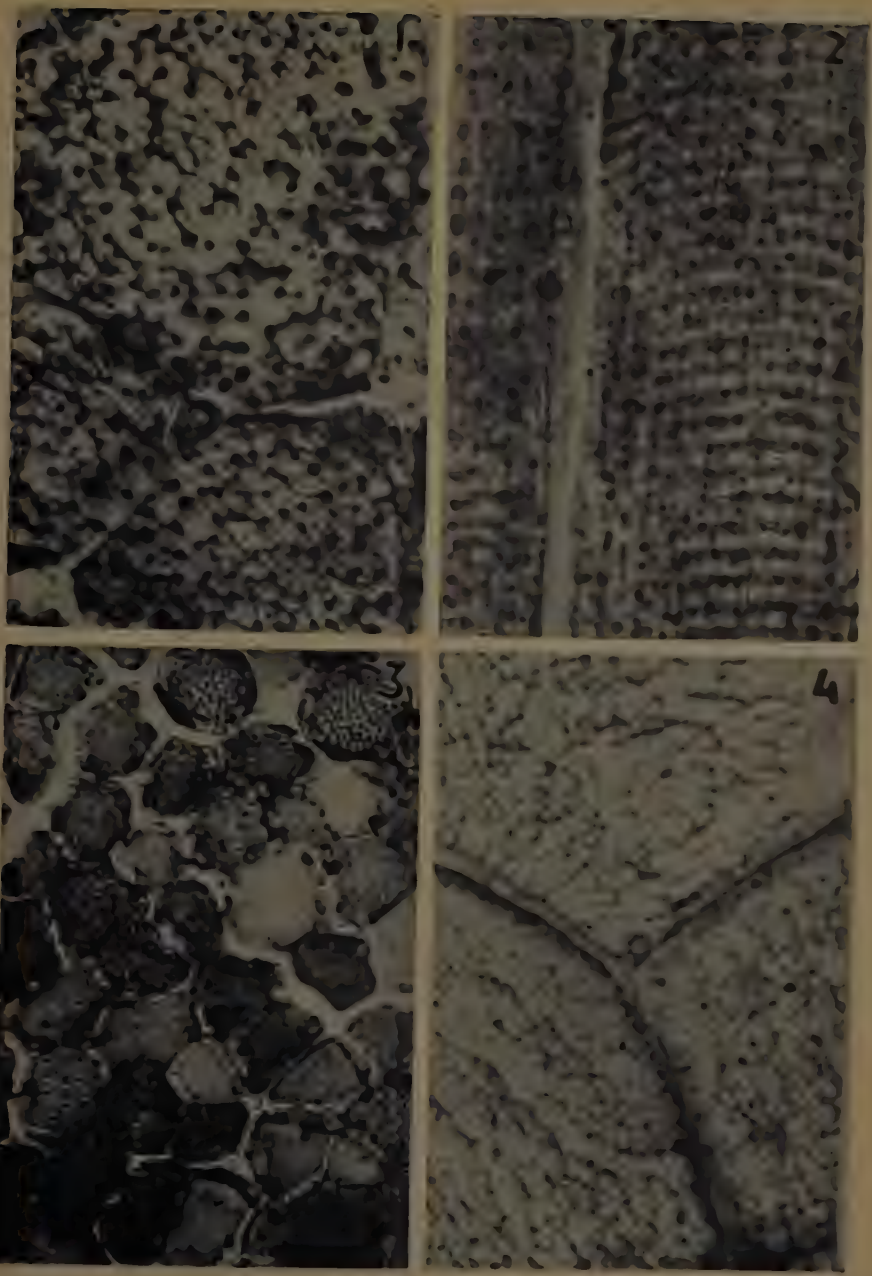

PLATE I

Fig. 1 T. s. of the kite pectoralis (superficial layer, Red fibres). Sudan Black B. x1700.

Fig. 2 L. s. of the kite pectoralis (superficial layer, Red fibres). Sudan Black B. x1700.

Fig. 3 T. s. of the parakeet pectoralis (superficial layer, Red and Light fibres) Sudan Black B. x450.

Fig. 4 T. s. of the fowl pectoralis (White fibres), Sudan Black B. x1700.

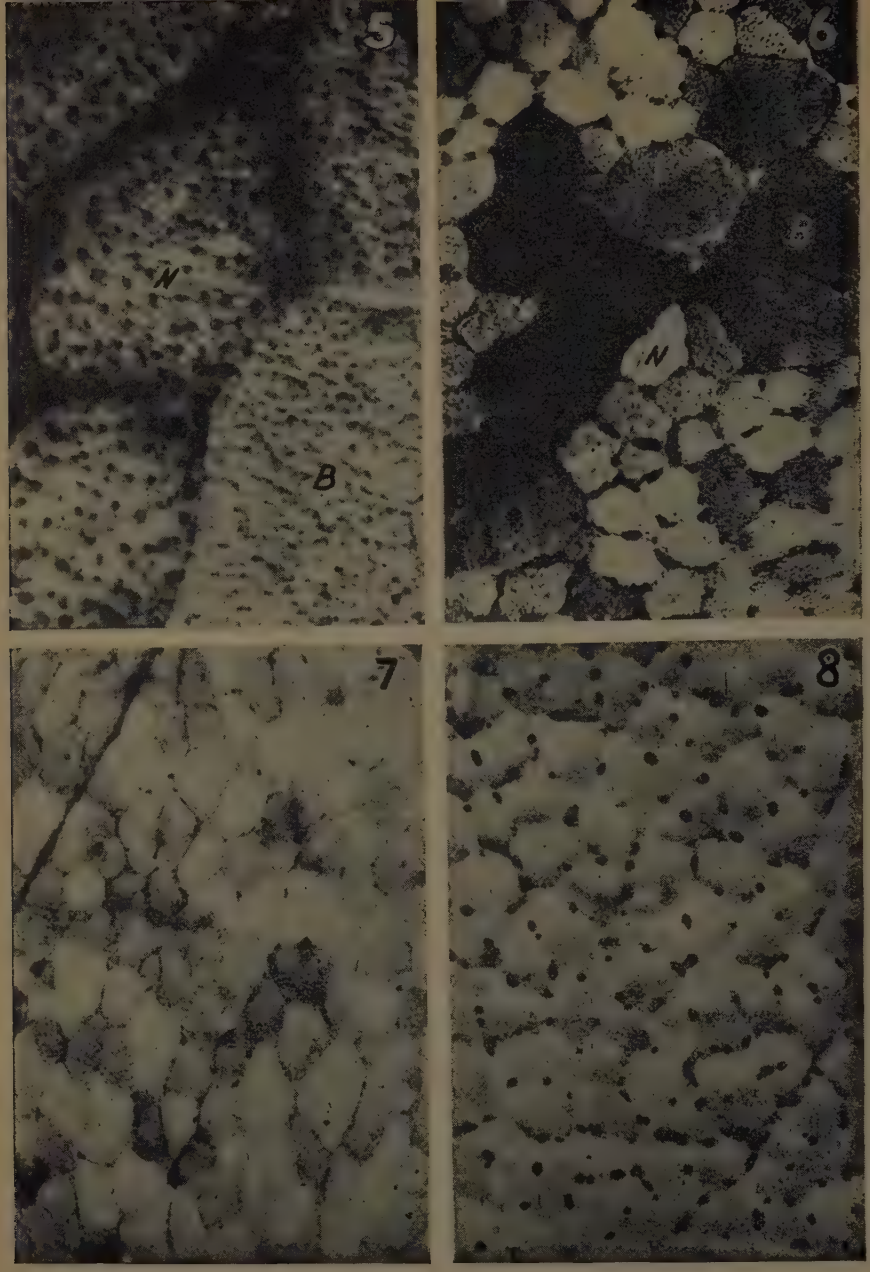

PLATE II

Fig. 5 T. s. of the dove pectoralis (Red and White fibres). (B.—Broad white fibre; N.—Narrow red fibre) Sudan Black B. x1700.

Fig. 6 T. s. of the dove pectoralis. (B.—Broad White fibres; N.—Narrow red fibre) PAS.—Hemalum. x450.

Fig. 7 T. s. of the kingfisher pectoralis. (Red and Light fibres) PAS—Hemalum. x450.

Fig. 8 T. s. of the parakeet pectoralis. PAS—Hemalum. x450.

Cytochemical study of glycogen shows that in the superficial part of the pectoralis of the kite, glycogen is uniformly distributed in all the fibres. Similarly, in the pectoralis of parakeet where a few light fibres (which are present in the superficial layer) differ from the dark fibres only slightly, glycogen is uniformly distributed in all the fibres (fig. 8). In the pectoralis of kingfisher the light and dark fibres are better differentiated and the fibres contain varying amounts of glycogen (fig. 7), but the relation between the size of the fibres and glycogen content is uncertain.

In the dove pectoralis, glycogen is concentrated in large amounts in the broad fibres whereas in the narrow fibres, it is comparatively much less (fig. 6).

Discussion

In the pectoralis with light and red fibres, the main differences between them are the higher mitochondrial density and the smaller fibre diameter in the red fibres. Paul and sperling (1952) have shown that the mitochondrial density bears a direct relation to the 'cyclophorase activity' in striated muscles. The reduction in the amount of succinic dehydrogenase with increase in fibre diameter has been noted in the mixed muscles of the rat (Nachmias and Padykula, 1958), the bat (George, Susheela and Scaria, 1958) and certain muscles of reptiles (Shah, unpublished). It appears that as differences in diameter between the red and light fibres increase, the associated differences in their structural and physiological make-up too, increase. The broad white fibres of the pectoralis of the dove contain negligible amount of mitochondria, whereas the narrow fibres are loaded with them. Similar observations have been made on the pectoralis of pigeon (George and Naik, 1958b). It has also been shown that in the narrow red fibres of the pigeon and dove the dehydrogenases activity is very high compared to the broad white fibres, in which it is almost negligible, (George and Scaria, 1958b and c).

With increase in the diameter of the fibres, the rate of diffusion of gases between blood capillaries and the middle part of the fibre should decrease. This could have been compensated for, were the blood capillaries surrounding the broad fibres be more in number than those surround the narrow fibres. But even in the pectoralis of the pigeon, the number of blood capillaries surrounding the broad fibres is about the same as those around the narrow fibres (George and Naik, unpublished).

The works of Green (1951) and George and Jyoti (1955 a and b)

have suggested the possibility that in the pectoralis of birds, fat might be oxidized directly during long and sustained activity and that the fat in muscle is not to be looked upon only as a reserve store which can be utilized for the production of energy when the occasion arises. George and Scaria (1958a) histo-chemically demonstrated higher lipase activity in the narrow fibres than in the broad fibres of the pectoralis of the pigeon. It appears that the processes leading to the synthesis and oxidation of fat are better developed in the narrow fibres than in the broad ones. On the other hand it has been shown by George and Naik (1958a, 1959) that there is a higher concentration of glycogen in the broad white fibres than in the narrow red fibres of the pectoralis of the pigeon. Same is the condition in the pectoralis of the dove. In other birds with mixed fibres in the pectoralis, no definite relation between the size of the fibres and glycogen content could be found. Nachmias and Padycula (1958) reported that in the biceps of albino rats the glycogen tends to be more abundant in the large fibres but they could not find a positive consistent correlation between the size of the fibres and the glycogen content. In most of the mixed muscles other than those of the pigeon and dove there is a great deal of overlapping in colour and fibre diameter between the red and light fibres and thus apart from the labile nature of the muscle glycogen, the difficulty in identifying the red and light fibres in sections treated with various reagents during the process of staining, makes the correlation between the distribution of glycogen and fibre diameter, difficult to establish.

This work brings forward once again the general relationship that exists between structure and function in animal tissues. In the fowl and the partridge the pectoralis which is little used, contains fibres poor in mitochondria. On the other hand, in all the active birds the pectoralis is primarily made up of red fibres rich in mitochondria.

The division of the pectoralis in the kite and vulture presents an interesting problem. Since the superficial layer appears physiologically more active than the deeper layer, it is quite possible that these two layers of the pectoralis of kite and vulture differ in their mode of action. The complete or incomplete separation of the pectoralis into superficial and deeper layers has been noted in several birds (Fisher, 1946; Fisher and Goodman, 1955 ; Jollie, 1957). It is not known whether, histologically these layers (described by the latter workers) resemble those of the pectoralis of the kite. However, Fisher (1946) from his work on the

locomotor apparatus of the vulture suggested the possibility of the deeper layer being a factor in the forward motion of the wing, to raise the anterior end of the wing and increase the angle of incidence when the bird is gaining altitude.

Summary

Structural differences based on fat, glycogen and mitocondrial content in the muscle fibres of the *pectoralis major* of some birds have been reported and an attempt has been made to correlate these differences with fibre diameter.

References

Baker, J. R. 1946 Histochemical recognition of lipines. *Quart. J. Micr. Sc.*, 87, 447.

Bennett, H. S. and Porter, K. S. 1953 An electron microscope study of sectioned breast muscle of the domestic fowl. *Am. J. Anat.*, 93, 61-105.

Clark 1947 As cited by Grey, P., in Microtomist's Formulary and Guide. Blackiston, Co. Inc., New York, 1954, p. 160.

Fisher, H. I. 1946 Adaptations and comparative anatomy of new world vultures. *Amer. Midland. Nat.*, 35, 545-728.

Fisher, H. I. and Goodman, D. C. 1955 Myology of the Whooping Crane, *Grus amcricana* Univ. of Illinois Press.

George, J. C. and Jyoti, D. 1955a Histological features of the breast and leg muscle of bird and bat and their physiological and evolutionary significance. *J. Anim. Morph. Physiol.*, 2 (1), 31-37.

George, J. C. and Jyoti, D. 1955b The lipid content and its reduction in the muscle and liver during long and sustained muscular activity. *Ibid.*, 2 (1), 38-45

George, J. C. and Naik, R. M. 1957a Studies on the structure and physiology of the flight muscles of birds. 1. Variations in the structure of the

Pectoralis major muscle of a few representative types and their significance in the respective modes of flight. *Ibid.*, **4**, 23-32.

George, J. C. and Naik, R. M. 1957b — Studies on the structure and physiology of the flight muscles of bats. 1. The occurrence of two types of fibres in the *Pectoralis major* muscle of the bat (*Hipposideros speoris*). Their relative distribution, nature of fuel store amd mitochondrial content. *Ibid.*, **4**, 96-101.

George, J. C. and Naik, R. M. 1958a — The relative distribution and the chemical nature of the fuel store in the two types of fibres in the *Pectoralis major* muscle of the pigeon. *Nature*, **181**, 709-710.

George, J. C. and Naik, R. M. 1958b — Relative distribution of mitochondria in the two types of fibres of the *Pectoralis major* muscle of the pigeon. *Nature*, **181**, 782-783.

George, J. C. and Naik, R. M. 1959 — Studies on the structure and physiology of the flight muscles of birds 4. Fibre architecture of the *Pectoralis major* of the pigeon. *Biol. Bull.*, **116**, 239.

George, J. C. and Scaria, K. S. 1958a — Histochemical demonstration of lipase activity in the *pectoralis major* muscle of the pigeon. *Nature*, **181**, 783.

George, J. C. and Scaria, K. S. 1958b — A histochemical study of dehydrogenase activity in the *pectoralis major* muscle of the pigeon and certain other vertebrate skeletal muscles. *Quart. J. Micr. Sc.*, **99**, 469.

George, J. C. and Scaria, K. S. 1958c Demonstration of succinic dehydrogenase in the breast muscle of the dove. (Under preparation for publication).

George, J. C., Susheela, A. K. and Scaria, K. S. 1958 Studies on the structure and Physiology of the flight muscles of bat. 3. Alkaline phosphatase and Succinic dehydrogenase activity in the the breast muscle—A Histochemical study. *J. Anim. Morph. Physiol.*, 5, 110-112.

Green, D. E. 1951 Cyclophorase system. Enzyme and Enzyme Systems. Edited by Edsall, J. T., Harward Univ. Press. p. 15-46.

Jollie, M. 1957 Avian anatomy and anatomist. *Condor*, 59, 394-397.

Nachmias, V. T. and Padykula, H. A. 1958 A histochemical study of normal and denervated red and white muscles of the rat. *J. Biophys. Biochem. Cytol.*, 4, 47-54.

Paul, M. H. and Sperling, E. 1952 Cyclophorase system XXIII. Correlation of cyclophorase activity and mitochondrial density in striated muscles. *Proc. Soc. Expt. Biol. Med.*, 79, 352-354.

Pearse, A. G. 1954 Histochemistry—Theoretical and Applied. J. and A. Churchill Ltd., London.

ON THE PRESENCE OF SPECIALIZED CONDUCTING (CONNECTING) TISSUE IN THE HEART OF THE INDIAN WALL LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

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According to Prakash (1953a, 1954a, b, 1957a, b, c) the specialized impulse-initiating and conducting tissue of the heart of lower vertebrates is a fore-runner of that of birds and mammals. Robb (1953) and Mori (1955) could not find any specialised conducting structures in the heart of turtles and crocodiles comparable to those of birds and mammals; but, however, expressed the view that the beginning of the avian and mammalian cardiac conducting system could be seen in reptiles. All these observations challange the neomorphic nature (Davies and Francis, 1946) of cardiac conducting tissue of birds and mammals and throw a new light on the phylogeny of impulse initiating and conducting structures of birds and mammals.

In the present investigation the heart of the lizard, *Hemidactylus flaviviridis*, has been examined with special reference to its conducting system.

Material and Method

Half a dozen wall lizards were collected and chloroformed. The hearts after being removed from chloroformed lizards were fixed in Bouin's picroformol. Serial sections, sagittal and coronal, 7 micra thick were cut. They were mounted and stained with acid fuchsin.

Observations

The heart consists of four chambers *viz.*, a sinus venosus, two atria and a ventricle. The sinus venosus is a thin-walled, irregularly-disposed chamber lying dorsally to the right atrium. It communicates with the right atrium through a sinuatrial opening and this opening is guarded by two sinuatrial valves.

Sinuatrial Node: An oval body, referred to as the sinuatrial node is present at the sinuatrial junction. It is a conspicuous structure, sur-

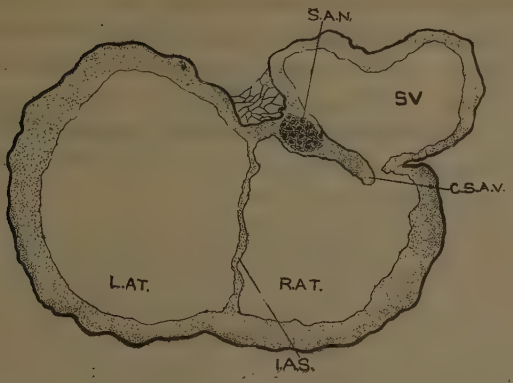

Fig. 1

Transverse section of the heart of *Hemidactylus* showing the sinuatrial node.

Fig. 2

Longitudinal section of the heart of *Hemidactylus* showing the atrioventricular bundle.

LIST OF ABBREVIATIONS

A. V. B.....Atrioventricular bundle.
C. S. A. V.....Caudal sinuatrial valve.
L. AT.....Left atrium
L. B.....Left branch of atrioventricular bundle
R. AT.....Right atrium
R. B.....Right branch of atrioventricular bundle
I. A. S.....Inter-atrial septum
S. V.....Sinus venosus
S. A. N.....Sinuatrial node
V.....Ventricle.

rounded by the wall of sinus venosus dorsally and by the right atrial wall ventrally. This structure possesses large cells with prominent nuclei and inter-woven fibres and can be well distinguished from the tissue that covers it all-round. It is histologically quite different from other tissues as it takes a deep stain with acid fuchsin. Because of the specialized histological nature of the fibres and the cells, of this tissue it has been referred to as sinuatrial node.

A study of serial sections of the heart, shows that the sinuatrial node is present just in continuity with and dorsal to, the caudal sinu-atrial valve. In those sagittal sections where the sinuatrial node has been seen the caudal valve does not appear completely, but a study of serial sections show that the sinuatrial valve is attached with to the sinuatrial node on one hand and to the interatrial septum on the other.

Therefore, it is clear that the cardiac stimulus of contraction which would be initiated at the sinuatrial node would go through the sinuatrial valve to the interatrial septum. From the interatrial septum it would be conveyed to both the atria.

Atrioventricular Bundle : Unrecorded so far and a very important feature of the heart of the wall lizard is the presence of the atrioventricular bundle (Text fig. 2) at the atrioventricular junction. This bundle is extensive, almost triangular in shape, with its apex attached to the caudal end of the interatrial septum and the base pointing towards the ventricular cavity. This structure is composed of a large number of prominent cells with large nuclei and here and there in between these cells are present interlaced fibres. These cells of the bundle are not as large as those of sinuatrial node, but are quite conspicuous and possess large perinuclear space. The bundle takes a deep and bright stain with acid fuchsin and is seen well marked from the surrounding tissue, thus indicating its histologically specialized nature like that of atrioventricular bundle (His, 1892) of birds and mammals. The base of the triangular bundle extends further laterally, attaching to the wall of the ventricle. These lateral extensions of the atrioventricular bundle may be called the right and left branches of the bundle.

The stimulus of contraction which would reach the interatrial septum from the sinuatrial node would be conducted to the atrioventricular bundle and from there it would pass to the ventricle through right and left branches of the bundle.

Thus there exists a definite path way for the conduction of stimulus of contraction from sinuatrial node to atrioventricular bundle through the interatrial septum.

Discussion

The non-existing histologically specialized impulse initiating and conducting structures of Davies and Francis (1946) have been observed in the heart of lizard, *Hemidactylus flaviviridis*. Davies and Francis denied the presence of specialized structures in the heart of lower vertebrates (salamander, crocodile and alligator). They arrived at the conclusion that the nodal and Purkinjie fibres of the heart of birds and mammals are neomorphic in nature. However, against Davies and Francis the present study confirms Prakash's description of distinct and well-defined specialized conducting structures in the heart of certain lower vertebrates. Now it seems certain as far as the histology of vertebrate heart is concerned that the nodal and Purkinjie fibres (Purkinjie, 1845) constituting the impulse initiating and conducting tissue of the heart of birds and mammals are neither neomorphic (Davies and Francis, 1946) in nature nor remnants (Kieth and Flack, 1907) of those of more extensive tissues of similar nature present in the heart of lower vertebrates; but have evolved and are further specializations (Prakash, 1957) of the tissue already existing for the purpose in the heart of lower vertebrates.

Summary

(1) Specialized impulse initiating and conducting tissue has been observed in the heart of *Hemidactylus flaviviridis*.

(2) A sinuatrial node has been observed at the sinuatrial junction.

(3) An atrioventricular bundle is present at the atrioventricular junction.

(4) The histologically specialized structure of the node and the bundle which resemble those of birds and mammals have been held to be responsible for initiation and conduction of the cardiac stimulus of contraction.

Acknowledgement

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References

Davies, F. and Francis, E. T. B.	1946	The conducting system of the vertebrate heart. *Biol. Rev.*, **21**, 173-188.
His, W.	1893	Die Thatigkeit des embryonalen Herzens und deren Bedentung fur die Lehre von der Herzbewegung beim Erwachsenen. *Arb med. Klin.*, Lpz., 14-50.
Kieth, A. and Flack, M.	1907	The form and nature of the muscle connection between the primary divisions of the vertebrate heart. *J. Anat.*, Lond., **41**, 172-189.
Mori, M.	1955	Atrioventricular system of crocodile heart. *Kyushu J. med. Sci*, **5**, 199-205.
Prakash, R.	1953	The heart of the common Indian catfish *Heteropneustis fossilis* (Bloch), with special reference to the conducting system. *Proc. Zool. Soc.*, Bengal, **6**, 113-119.
Prakash, R.	1954a	The heart and its conducting system in the tadpoles of frog, *Rana tigrina* (Daudin). *Proc. Zool. Soc.*, Bengal, **7**, 27-37.
Prakash, R.	1954b	Evolution in the conducting system of the vertebrate heart. *Ind. Med. J.*, July, 164-165.
Prakash, R.	1957a	On the significance of the atrioventricular valves of the tadpoles of *Rana tigrina* (Daudin) *J. Vikram Univ.* India, **1**, 94-95.
Prakash, R.	1957b	On the function of the spiral valve in the heart of the tadpole of *Rana tigrina*. *Sci. & Cult.*, **22**, 567-568.
Prakash, R.	1957c	The atrioventricular bundle in the heart of the banded krait, *Bangarus*

fasciatus. Proc. Nat. Inst. Sci., India, **22**, B, 255-258.

Purkinjie, J. E. 1845 Mikroskopisch-neurogische Beobachtungen. *Arch. Anat. Physiol. U. Wissench.*, Med. **281**.

Robb, J. S. 1953 Specialised (conducting) tissue in the turtle heart. *Amer. J. Physiol.*, **171**, 7-13.

FURTHER HISTOCHEMICAL OBSERVATIONS ON THE ADIPOSE TISSUE OF THE PIGEON

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OUR earlier studies on the adipose tissue of the pigeon has revealed the presence of a number of enzymes and a profuse supply of blood in this tissue. Lipase and alkaline phosphatase were shown to be occurring in appreciable concentrations (George and Eapen, 1958). Lipase plays an important role in lipid metabolism. The presence of alkaline phosphatase in the adipose tissue appears to be connected with lipid metabolism. This is significant due to the fact that the pigeon is known to utilize chiefly fat for sustained muscular activity (George and Jyoti, 1957). The aforesaid enzymes are reported to be present only in the fat-loaded narrow fibres of the pigeon breast muscle and not in the glycogen-loaded broad ones (George and Scaria, 1958 ; George *et al*, 1958). Here we report the results of certain histochemical studies on the adipose tissue of the pigeon *(Columba livia)*. The adipose tissue was tested for the following: adenosine triphosphatase (ATPase), acid phosphatase, glycogen, fat, phospholipids, cholesterol, sulphydryl groups and water-insoluble aldehydes and ketones.

Material and Methods

The material used in the present study was the visceral adipose tissue occurring in association with the small intestine in the pigeon *(C. livia)*. The birds were killed by decapitation and the tissue taken after most of the blood was drained off.

The presence of ATPase was tested by employing the method of Pearse and Reis (Pearse, 1954) using adenosine triphosphate (ATP) as substrate. Fresh frozen sections were mounted on clean, dry slides without any adhesive and left at room temperature for about 15 minutes to ensure adherence. The sections were then defatted in ethyl ether at room temperature for 10 minutes and transferred to 10% neutral formalin for fixation at 4°C. One hour fixation was followed by washing in cold running water for 1 hour and incubation in the substrate medium

for 4 hours at 37°C. The incubation medium used was the same as described by Pearse (1954). After incubation the sections were rinsed in 2% calcium nitrate, thoroughly washed with distilled water and treated with 2% cobalt nitrate solution for 5 minutes. They were again washed well with distilled water and treated with dilute yellow ammonium sulphide solution. The following four controls were tried: Alkaline phosphatase at pH 7.5 and 9.2, ATP blank and sections which were kept in boiling water for 10 minutes prior to incubation.

Acid phosphatase was tested according to the method of Gomori (Glick, 1949) using sodium glycerophosphate as substrate. Defatted sections were fixed in 10% formalin in cold (4°C) for 1 hour, washed for 1 hour in running water and finally incubated for 6 hours at 37°C. The incubation medium was the same as described by Glick (1949). The stock solution of Glick was diluted with twice the amount of distilled water, filtered and used for incubation. The procedure was the same as described by Glick (1949). Two controls were tried: Sections kept in boiling water for 10 minutes before incubation and sections incubated in an incubation medium devoid of glycerophosphate.

Paraffin sections of adipose tissue treated with Lison and Voaker fixative were stained for glycogen by the Best's carmine method (Pearse, 1954). Sections stained after removal of gycogen with saliva served as control.

For staining of fat, the tissue was first fixed in Calcium-formal and then 15μ gelatin sections were taken and stained with saturated Sudan black B in 70% ethanol. Cain's method (Pearse, 1954) for the demonstration of neutral and acidic lipids were also employed.

Baker's acid haematin method was employed for the detection of phospholipids (Pearse, 1954). Pyridine extracted sections served as control (Pearse, 1954).

Cholesterol detection was according to the Schultz method (Pearse, 1954). Bourne nitroprusside method for sulphydryl groups and Albert and Leblond method for water-insoluble aldehydes and ketones (Glick, 1949) were successfully conducted.

Results and Discussion

From the intensity of staining, it was found that ATPase and acid phosphatase occur in the cell periphery as well as in the nuclei in considerable concentrations. Zimny and Gregory (1958) investigating the

composition of the brown adipose tissue of the 13-striped ground squirrel has reported the concentration of the following phosphate compounds in a decreasing gradation : inorganic phosphate, creatine phosphate and adenosine triphosphate. There are no data available as far as we can gather, on the presence or concentration of these different phosphate compounds in the adipose tissue of the pigeon or any other bird. The role of ATPase present in a fair concentration in the pigeon adipose tissue cannot, even be suggested at the present state of our knowledge. The same could be said about acid phosphatase too.

Glycogen was not found in the adipose tissue. Fawcett (1952), however, reported the presence of a considerable quantity of glycogen in the brown adipose tissue of rats and a negligible amount in the yellow type. He (1952) has further stated that rats which were fasted for 24 hours and then fed, showed an increase in the glycogen content in the adipose tissue when examined after 2 to 12 hours from the commencement of feeding. In a similar procedure we examined a number of pigeons which were made to fast for 24 hours and at the end of that period they were fed with a diet consisting of cereals. The birds were sacrificed at different time intervals of 2 hours, 4 hours and 8 hours from the time of termination of the fasting and their adipose tissue was examined for glycogen. Negative results were obtained in all the cases. Diurnal variations in the levels of glycogen in the adipose tissue were also noted by Fawcett (1952). Rats which were killed and examined during night hours revealed a greater concentration of glycogen than others taken during day time (Fawcett, 1952). In our present study all the pigeons used were sacrificed during day time.

Sections stained with Sudan black B revealed that the cell periphery contains negligible amounts of fat, while towards the centre of the cell, is found a single, large fat droplet. This is in conformity with Fawcett's (1952) observation on the fat in the yellow adipose tissue of rats. Sections stained with Nile blue showed a predominant red colouration indicating the abundance of neutral fats. Acid lipids were absent, as there was no blue colouration anywhere. Phospholipids and cholesterol were found only in very low concentrations. From these results it appears that the lipids in the adipose tissue of the pigeon are chiefly in the form of neutral fats.

Sulphydryl groups and water-insoluble aldehydes and ketones were

also not found in appreciable concentrations. It should be of interest to note here that the brown adipose tissue of the bat contains considerable quantities of sulphydryl groups, and the yellow variety a small amount (George and Eapen, 1959).

Summary

The presence of ATPase, acid phosphatase, glycogen, fat, phospholipids, cholesterol, sulphydryl groups and water-insoluble aldehydes and ketones in the adipose tissue of the pigeon was tested by histochemical methods. ATPase, acid phosphatase and neutral fats were found in appreciable quantities, while phospholipids, cholesterol, sulphydryl groups and water-insoluble aldehydes and ketones were found to be present only in low concentrations. Sections treated to show glycogen gave negative results.

References

Fawcett, D. W.	1952	*J. Morph.* **90**, 363.
George, J. C. and Eapen, J.	1958	*J. Anim. Morph. Physiol.* **5**, 49.
George, J. C. and Eapen, J.	1959	*Quart. J. Micro. Sci.* (In Press)
George, J. C. and Jyoti. D.	1957	*J. Anim. Morph. Physiol.* **4**, 119
George, J. C., Nair, S. M. and Scaria, K. S.	1958	*Curr. Sci.* **27**, 172.
George, J. C. and Scaria, K. S.	1958	*Nature*, **181**, 783.
Glick, D.	1949	Techniques of Histo and Cyto-chemistry. (Interscience Publishers, Inc. New York).
Pearse, A. G. E.	1954	Histochemistry, Theoretical and Applied. (J. & A. Churchill Ltd. London).
Zimny, M. L. and Gregory, R.	1958	'Paper read by title' Annual session of American Association of Anatomists. *Anat. Rec.* **130**, 390.

STUDIES ON THE OXYGEN CONSUMPTION IN TROPICAL POIKILOTHERMS

III. Oxygen consumption in the fresh water fish, *ETROPLUS MACULATUS* (BLOCK) in relation to size and temperature

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IN recent years much attention has been paid to studies on respiration in poikilotherms (Scholander, Flagg, Walters and Irving, 1953; Rao and Bullock, 1954; Bullock, 1955; Zeuthen, 1953, 1955). Most of these studies were concerned with arctic and temperate forms. Moreover in a good number of these studies marine animals were used to investigate the effects of body size and temperature on respiration. Freshwater poikilotherms, particularly those of the tropics, have not received as much attention. This is all the more true of fishes, on which there are few studies on the effects of body size and temperature on respiration. In tropical poikilotherms acclimatization to temperature is a topic, which has been little studied. Before any investigations in that direction are taken up, it will be profitable to have a clear understanding of the effects of temperature and body size on the oxygen consumption of these poikilotherms. It is with this intention in view that the present investigations have been taken up.

Materials and Methods

Etroplus maculatus were collected from the freshwater tanks at Kalahasti, a place about 25 miles north of Tirupati. The fish were kept in the laboratory in a large aquarium tank along with some other varieties of fish like *Barbus sp.* The temperature of the aquarium varied between 29 and 30°C during the period of these studies. Water was changed in the aquarium once a week and the fish were regularly fed once in two days. Studies were made on two fish at a time, which were kept in separate respiration chambers of the type reported by Saroja (1959) and their oxygen consumption measured at different temperatures, namely, 15°C, 30°C, and 35°C. Measured samples of water were obtained from the respiration chambers, both, before the start and after the experiment. The oxygen content of the samples was determined by the direct Winkler's iodometric method as given in Welsh and Smith (1953). The difference in the oxygen content of the initial and final samples in each experiment

will give the amount of oxygen consumed by the fish during the experimental period (30 minutes). Similar measurements were taken for a number of fish of different body weights. For measuring the oxygen consumption at 15°C the experiments were set up inside a refrigerator and at 35°C, inside a thermostatically controlled hot bath. Immediately after each experiment, the fish studied was killed and its wet weight taken after carefully blotting out the water on its body. The respiration chambers were kept airtight and free from air bubbles during the experimental period. Also care was taken to keep the experimental temperature constant ($\pm$ 1°C). The outside of the respiration chambers was painted black to keep their interior dark, so as to keep the activity of the fish minimised. Individual fish were isolated and kept without any food for about 24 hours before they were experimented upon. The fish did not produce any slime.

Results

The data obtained are summarised in Table 1 and the same plotted in several graphs.

TABLE 1

Data of total oxygen consumption in *Etroplus maculatus* of different body weights at different temperatures

Serial number	Weight of fish Gms.	O_2 ml./hr.		
		15°C	30°C	35°C
1	9.4	0.306	0.857	0.980
2	7.4	0.352	0.612	0.765
3	5.5	0.184	0.536	0.704
4	5.3	0.199	0.475	0.689
5	5.3	0.184	0.704	0.780
6	4.2	0.155	0.397	0.532
7	3.4	0.087	0.329	0 484
8	2.6	0.097	0.310	0.426
9	2.5	0.145	0.387	0.455
10	2.4	—	0.206	—
11	2.3	0.092	0.505	0.398
12	1.7	—	0.348	—
13	1.1	0.039	0.329	0.166
14	1.0	0.058	0.126	0.203
15	1.0	0.030	0.153	—
16	1.0	0.058	0.116	—
17	0.9	0.092	0 107	0.229
18	0.6	—	0.168	—
19	0 6	—	0.136	—
20	0.5	0.020	0.116	—

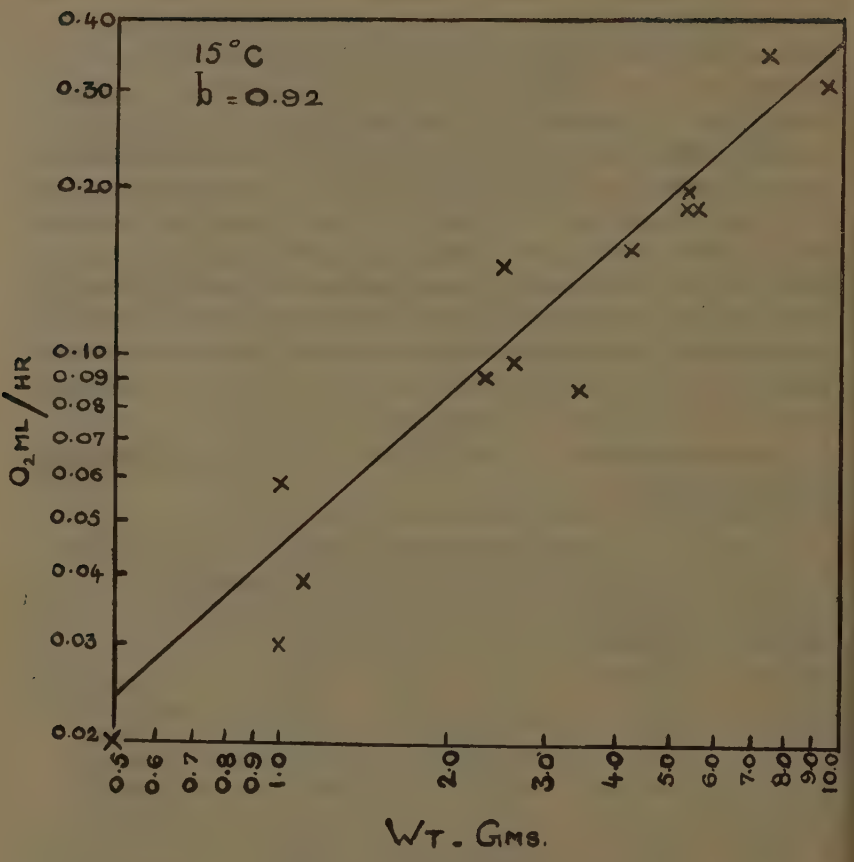


Fig. 1 (a)

Oxygen consumption as a function of body size :

A study of the data presented in Table 1 reveals that the oxygen consumption of *Etroplus maculatus* increases with the increasing body weight at all temperatures studied. At any given temperature the oxygen consumption per unit time is lesser in the smaller fish than in the larger ones. The degree to which there is such an increase in the total oxygen consumption with increasing body weight is not the same at all temperatures studied, as can be seen from the regression coefficients of the size-metabolism curves presented in Fig. 1. The weight specific QO_2 of fish of representative weights were calculated from the curves of Fig. 1 and the

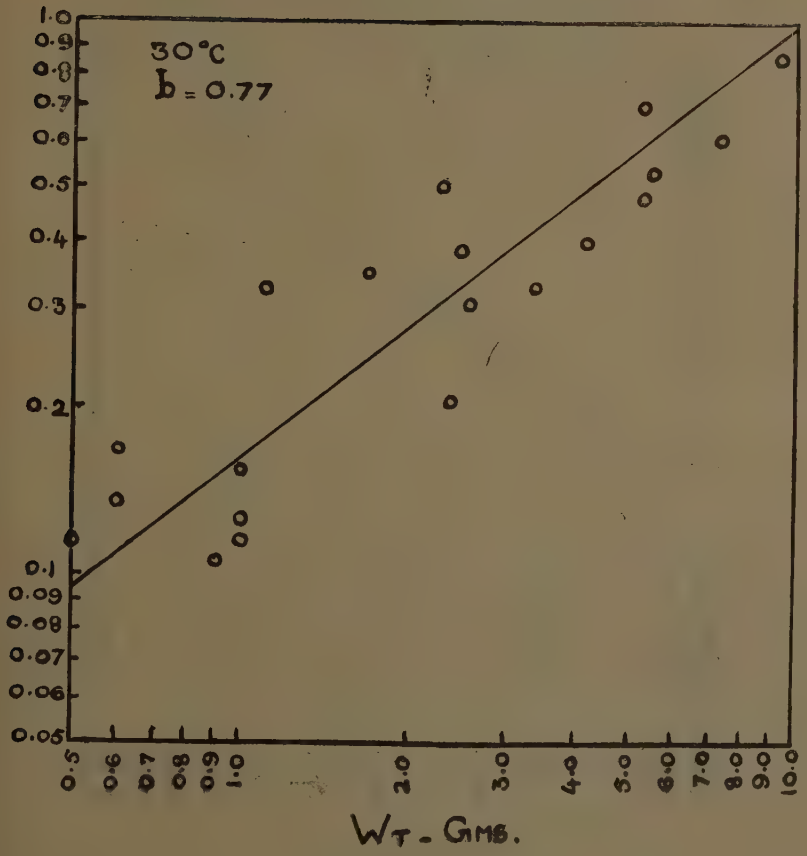


Fig. 1 (b)

same presented in Table 2. These values indicate that the unit oxygen consumption (O_2 ml./gm./hr.) decreases with the increasing body weight of the fish, such a decrease being comparatively more conspicuous at 30 and 35°C than at 15°C, thus reflecting what has been noted with regards to the total oxygen consumption (O_2 ml./hr.).

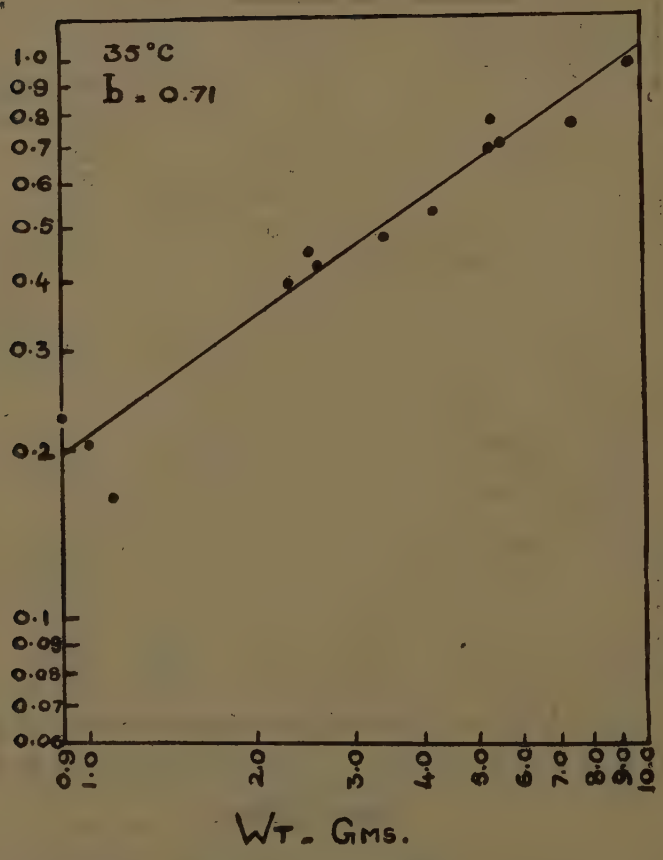


Fig. 1 (c)

LEGENDS TO FIGURES

Fig. 1—Total Oxygen consumption in *Etroplus maculatus* as a function of body size at different temperatures, plotted on double logarithmic grid. The points in the figure represent individual measurements.

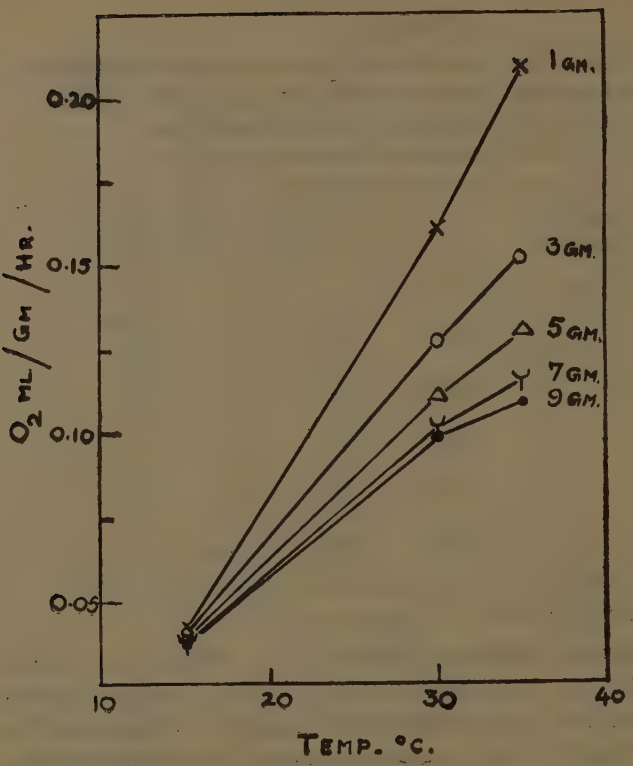
TABLE 2

Oxygen consumption per gram of body weight per hour in *Etroplus maculatus* of different weights at different temperatures. The values are calculated from the size-metaboiism curves of Fig. 1.

Weight of fish Gms.	O_2 ml/gm./hr.		
	15°C	30°C	35°C
1	0.043	0.162	0.209
3	0.041	0.127	0.152
5	0.039	0.112	0.129
7	0.038	0.103	0.116
9	0.038	0.098	0.109

Oxygen consumption as a function of temperature :

The data given in Table 2 are plotted as Rate-Temperature curves in Fig. 2. A study of these curves for fish of different weights suggests that while all the fish studied respond to changes in temperature by increasing or decreasing their oxygen consumption, such a temperature response is highly size dependent. The smaller fish are much more responsive to temperature changes than the larger ones. The same trend can also be made out from the varying b values of the size-metabolism curves presented in Fig. 1. The b values decrease with increasing temperature. Thus at 15°C the b value of the curve is 0.92, at 30°C it is 0.77 and at 35°C it is 0.71, indicating that the increase in the oxygen consumption in larger fish with increasing temperature is lesser than in the smaller fish, which, hence, are to be regarded as being more sensitive to temperature changes than the larger ones. The same can be made out from the data of Table 2 and the R-T curves presented in Fig. 2 as also from the Q_{10} values discussed below. Thus in a 1 gm. fish the weight specific QO_2 rises from 0.162 to 0.209, when the temperature is raised from 30 to 35°C and drops from 0.162 to 0.043 when the temperature is lowered from 30 to 15°C. The corresponding rise or fall in the values for a 9 gm. fish is much lesser.



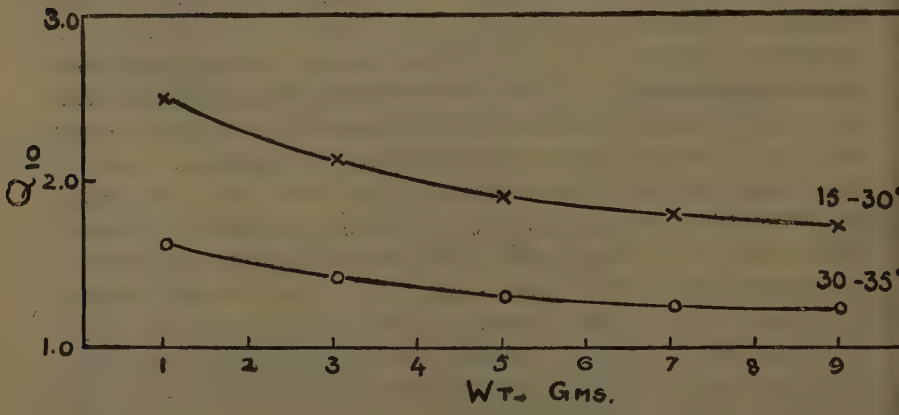


Fig. 2—Rate (O_2 ml/gm./hr)—Temperature curves of *Etroplus maculatus* of 1, 3, 5, 7, and 9 gm. body weights. The points are calculated from the curves of fig. 1.

Fig. 3—Q_{10} of oxygen consumption in *Etroplus maculatus* as a function of body weight at different temperature ranges. The Q_{10} values are calculated from the R-T curves presented in fig. 2.

TABLE 3

Q_{10} of oxygen consumption in *Etroplus maculatus* in relation to body size and temperature. The Q_{10} values are calculated from the data presented in Table 2

Weight of fish Gms.	15-30°C.	30-35°C.
1	2.51	1.66
3	2.07	1.44
5	1.91	1.32
7	1.81	1.27
9	1.72	1.24

Q_{10} as a function of body size and temperature:

The Q_{10} of oxygen consumption for the fish of various weights at various temperature ranges studied, was calculated from the data of Table 2 and the same presented in Table 3 and plotted in Fig. 3. In *Etroplus maculatus* the Q_{10} systematically decreases with increasing body weight at all temperature ranges studied, this trend being much more conspicuous at the lower temperature range like 15-30°C than at the higher like 30-35°C. At both these temperature ranges the decrease in the Q_{10} values with increasing body size is much more marked in fish of smaller weight than in those of larger weight. Thus between 1 and 3 gm. fish the Q_{10} drops from 2.51 to 2.07 between 15 and 30°C and from 1.66 to 1.44 between 30 and 35°C, while the corresponding values between 7 and 9 gm. fish are from 1.81 to 1.72 and from 1.27 to 1.24 respectively. Even in these smaller fish the decrease in the Q_{10} with increasing body size is more steep at the lower of the two temperature ranges studied. It may also be noted from Table 3 and Fig. 3 that the Q_{10} of oxygen consumption decreases with increasing temperature in all fish regardless of weight and such a decrease is again, more marked in smaller fish than in larger ones. Thus between 15 and 30°C and 30 and 35°C the decrease in the Q_{10} in a 1 gm. fish is from 2.51 to 1.66, while in a 9 gm. fish the corresponding decrease is only from 1.72 to 1.24. Thus the

smaller fish have higher Q_{10} values at all temperature ranges studied—lower as well as higher—suggesting that they are much more sensitive to temperature changes than the larger ones.

Discussion

The metabolism of *Etroplus maculatus* shows an upward trend with the increase of its body size. The straight lines of the size-metabolism curves indicate that throughout the weight range of the fish studied the oxygen consumption increases with the same power of the body weight at any given temperature. (Fig. 1). Conversely the unit oxygen consumption decreases with increasing body weight, this being most marked at 35°C and becoming less and less so at lower temperatures. (Table 2). At 35°C between 1 and 9 gm. fish the unit oxygen consumption decreases from 0.209 to 0.109, while the corresponding decrease at 30°C and 15°C is only from 0.162 to 0.098 and from 0.043 to 0.038 respectively. The unit oxygen consumption values of various fish at 30°C considered in relation to those at the higher and lower temperature extremes would readily indicate that amongst the fish studied the smaller ones are more temperature sensitive than the larger ones. The habitat temperature and the temperature gradient have considerable influence on the temperature tolerance capacities of these fish, as can be seen from the extent of displacement of the size-metabolism curves between 30 and 35°C and between 30 and 15°C, as also the varying slopes of these curves at these temperatures. Thus the widest displacement of the curves is between 30 and 15°C and the curve at 15°C has the maximum slope. 15°C is a temperature, which normally the tropical fresh water fish are not subjected to and hence at this unusually low temperature the metabolic rate of the smaller fish is lowered much beyond the level that normally accompanies the lowering of temperature. As such the smaller fish appear to be less tolerant of 15°C, where they show indications of cold depression. On the other hand 35°C is not completely new to these fish as during summer months their habitat temperature may rise nearly to that level. But in another freshwater fish, *Barbus sophore*, the smaller fish were found to be sensitive as well as tolerant of these temperature extremes (15 and 35°C) unlike the larger ones, which show marked heat depression at 35°C and indications of cold depression at 15°C. (Parvatheswararao, 1959).

The regression coefficients of the size-metabolism curves are shown to be temperature dependent, decreasing with increasing temperature.

Obviously this is correlated with the greater responsiveness on the part of the smaller fish to the increase or decrease of temperature on either side of the norm. When the temperature is lowered to 15°C the size-metabolism curve towards the lower weight ranges of the fish is pulled down due to a greater metabolic decrease in smaller fish than in the larger ones resulting in a steep curve and when temperature is raised to 35°C the corresponding part of the curve is raised up due to a greater increase in metabolism in the smaller fish than in the larger ones resulting in a less steep curve. Accordingly, the smaller fish have higher Q10 values at these two temperature ranges, higher as well as lower. (Table 3). Likewise job's data on Milk fish, *Chanos chanos* (1957) showed higher regression coefficients for the size-metabolism curves at 25°C than for those at 29°C, as smaller fish were found to be more responsive to the lowering of temperature. In the common Indian earth worm, *Megascolex sp*, Saroja (1959) has shown that the *b* values of the size-metabolism curves decreased with increased temperature. On the other hand in *Barbus sophore* it was found that the *b* values decreased with temperature on either side of the habitat temperature (15-35°C). (Parvatheswararao, 1959). In several instances amongst fishes, it *was* shown that the proportionality of oxygen consumption was intermediate between surface area dependence and weight dependence (Fry, 1957). A similar trend is to be noted in the present case also, where, at the habitat temperature the *b* value of the size-metabolism curve is 0.77, indicating such an intermediate condition.

It can be noted from Table 2 and also Figure 2 that the decrease in the unit oxygen consumption with increasing body size in *Etroplus maculatus* is greater in smaller fish than in the larger ones at any given temperature. Identical trends in the unit oxygen uptake were shown by Eliassen (1952) in *Artemia salina*, where the rate of decrease in the unit oxygen uptake was found to be greater in the case of nauplli than in the middlesized animals.

That the smaller fish of *Etroplus maculatus* are much more sensitive to temperature changes than the larger ones is indicated by their higher Q_{10} values at all temperature ranges studied (Table 3). At 15°C the smaller fish have a high Q_{10} value and this is probably suggestive of the smaller fish being cold depressed at this unusually low temperature for a tropical fresh water fish. But at 35°C, the smaller fish, though having a higher Q_{10} value, did not show any indication of heat depression. Unlike this in the fresh water fish, *Barbus sophore* the larger fish were found to

be markedly heat depressed at 35°C (Parvatheswararao, 1959). In several tropical fish studied by Scholander *et al* (1953) heat depression at 35°C was noted only in a couple of cases, *Abudefduf saxatilus* and *Haemulon bonariense*. The Q_{10} values calculated from Job's data on the milk fish, *Chanos chanos* (1957) show that, as in the present case, the smaller fish are more temperature sensitive than the larger ones, which have lower Q_{10} values.

At all temperatures studied the Q_{10} of oxygen consumption in *Etroplus maculatus* is clearly size dependent, decreasing with increasing body weight. This size dependence, further, is more marked at lower temperature range than at the higher and this is possibly correlated with the probable setting in of cold depression in smaller fish at 15°C. Thus at 15 and 30°C range the Q_{10} drops, between 1 and 9 gm. fish, from 2.31 to 1.72, while at 30 and 35°C range the corresponding drop is only from 1.66 to 1.24. In the case of *Barbus sophore* the Q_{10} values were found to be more size dependent at higher temperature ranges than at the lower. (Parvatheswararao, 1959). It may further be made out from Table 3 that the decrease in the Q_{10} with increasing body size is not uniform throughout the weight range of the fish studied, but much more steep and conspicuous at the lower weight ranges than at the higher. (Fig. 3)

In *Entroplus maculatus* Q_{10} decreases with increasing temperature (Table 3). But such a decrease is much more marked in smaller fish than in the larger ones, thus suggesting that the smaller fish are much more sensitive to temperature changes than the larger ones. The smaller fish appear to be intolerant of the 15°C, which is normally unusual in the tropics, and hence show indications of cold depression, as indicated by the high Q_{10} value of the 1 gm. fish at 15-30°C, as against that at 30-35°C. A similar trend, as in the present one, has been noted in the case of the limpet, *Acmaea limatula*, where the Q_{10} of heart rate decreases with increasing temperature (Segal, 1956). Reviewing the literature on Q_{10}, Rao and Bullock (1954) have shown that in a good number of cases amongst poikilotherms the Q_{10} increases with increasing body weight within the physiologically normal temperature ranges and they have also pointed out cases, where the Q_{10} decreases with increasing body weight, the latter trend being uncommon. There is not much information available on fishes in this direction except, possibly, for the works of Job (1955) on *Salvelinus fontinalis* as reported by Fry (1957), and Wells (1935) on *Fundulus parvipennis*. Job's data on the active metabolism

in *Salvelinus fontinalis* show that the slope of the size-metabolism curves becomes less with increasing temperature so that larger fish have lower Q_{10} values than the smaller ones, as in the present case. Wells' data on the whole indicate a decrease in the Q_{10} with increasing body size. But the Q_{10} values calculated from his data show (at 10-22°C) a decrease with increasing weight in two experiments and slight increase with increasing weight in a third experiment, using the same fish in all the three experiments. The temperature range as well as the weight range of the fish studied by Wells are too limited to allow for any generalisations in this regard. In the present studies on *Etroplus maculatus* a much wider temperature range as well as weight range of the fish have been made use of and the results show that in the present case Q_{10} decreases with increasing body size of the fish at all temperatures and in all fish the Q_{10} decreases with increasing temperature. In view of the paucity of information about the Q_{10} of oxygen consumption in fishes, studies on a greater variety of fish would be of extreme value.

Acknowledgements

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Summary

1. Oxygen consumption in *E. maculatus* increases with increasing body weight within the range studied (0.5 to 9.4 gms.). The rate of this increase varies with temperature within the range studied (15 to 35°C). The regression coefficient of the size-metabolism curves decreases with increasing temperature.
2. The unit oxygen consumption increases with decreasing body weight and the rate of this increase varies with temperature. The smaller fish are more sensitive to temperature changes than the larger ones.

The smaller fish appear to be less tolerant of lower temperatures like 15°C, where they show indications of cold depression.

3. Increased temperature results in increased oxygen uptake. The Q_{10} decreases with increasing temperature, such a decrease being more marked in smaller fish than in the larger ones. The smaller fish have higher Q_{10} values at all temperatures.

4. At all temperature ranges studied the Q_{10} decreases with increasing body size, such a decrease being more marked at lower temperatures than at the higher.

References

Bullock, T. H. 1955 Compensation for temperature in the metabolism and activity of poikilotherms. *Biol. Rev.*, **30**, 311-342.

Eliassen, E. 1952 The energy metabolism of *Artemia salina* in relation to body size, seasonal rhythm and different salinities. *Univ. Bergen Arbok. Naturvitenskap. Rekke.*, **11**, 1-17.

Fry, F. E. J. 1957 *The Physiology of fishes.*, Vol. 1., Academic Press Inc., New York.

Job, S. V. 1957 The routine-active oxygen consumption of the milk fish. *Proc. Ind. Acad. Sci.*, **45** (6), Sec. B., 302-313.

Parvatheswararao, V. 1959 Studies on the oxygen consumption in tropical poikilotherms : IV. Oxygen consumption in the fresh water fish, *Barbus sophore* in relation to size and temperature. (in Press).

Rao, K. Pampapathi and Bullock, T. H. 1954 Q_{10} as a function of size and habitat temperature in poikilotherms. *Amer. Nat.*, 78, 33-44.

Saroja, K. 1959 Studies on the oxygen consumption in tropical poikilotherms : II. Oxygen consumption in relation to body size and temperature in the earth worm, *Megascolex mauritii* when

kept submerged under water. *Proc. Ind. Acad. Sci.*, Sec. B., 49(3), 183-193

Scholander. P. F., W. Flagg.; V. Walters., and L. Irving 1953 Climatic adaptation in arctic and tropical poikilotherms. *Physiol. Zoöl.*, **26**, 67-92.

Segal, E. 1956 Microgeographic variation as thermal acclimation in an intertidal mollusc. *Biol. Bull.*, **3**, (1), 129-152.

Wells, N. A. 1935 The influence of temperature upon the respiratory mechanism of the pacific killifish, *Fundulus parvipennis*. *Physiol. Zoöl.*, **8**, 196-227.

Welsh, J.H. and R.I. Smith 1953 *Laboratory exercises in invertebrate physiology*. Burgess Publishing Co. Minneapolis.

Zeuthen, E. 1953 Oxygen uptake as related to body size in organisms. *Quart. Rev. Biol.*, **28** (1), 1-12.

Zeuthen, E. 1955 Comparative Physiology (Respiration). *Ann. Rev. Physiol.*, **17**, 459-482.

A COMPARATIVE STUDY OF THE OXYGEN-CARRYING CAPACITY OF THE BLOOD IN WATER- AND AIR-BREATHING TELEOSTS

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THE oxygen-carrying capacity of the blood of a number of teleosts has been determined by several researchers including Hall and Gray (1929), Root (1931), Redfield (1933), Willmer (1934) and Black (1940). The results obtained were based on the amount of iron present in the blood, or were arrived at by direct measurement of the oxygen in the blood by using Van-Slyke's manometric apparatus. The figures of Hall and Gray for Bonito and Mackerel are the highest and they attributed them to the active habits of the fishes. Some of the fresh-water fishes met within swamps containing acidic water have a higher oxygen-carrying capacity of blood (Black, 1940). So far no investigations have been undertaken to determine the comparative oxygen-carrying capacity of the blood in water and air-breathing teleosteans. In this paper are reported the results obtained from a comparative study of the oxygen-carrying capacity of the blood in a number of water and air-breathing teleostean fishes.

The oxygen-carrying capacity of the blood primarily depends on its haemoglobin content. Since the amount of iron present in the blood is a measure of the quality of the haemoglobin present in terms of its oxygen-carrying capacity, the comparison has been made by determining the amount of iron in milligrams in 100 cc. of blood. The method adopted for the determination of iron in the blood is that of Kennedy, using the Klett-Summerson photo-electric calorimeter in the final stages. Necessary precautions were taken during the investigations. The fishes were allowed to settle to normal conditions before attempting to extract the blood. Only venous blood extracted from the ventricle with the aid of a syringe previously washed with 5% neutral potassium oxalate solution was used for analysis.

Results

At least 6 readings were recorded for each fish.

The amount of iron determined in mg. per 100 cc. of the blood in the fishes investigated is as follows :—

	Name of the fish	The amount of iron estimated	
		Minimum	Maximum
	Water-breathing fishes		
1	*Glyphidodon caelestinus* (Cuv. & Val.)	17.80	26.87
2	*Labeo rohita* (Ham.)	15.13	19.83
3	*Catla catla*	24.46	27.52
4	*Cirrhina mrigala* (Ham.)	18.38	24.31
5	*Tetrodon patoca* (Ham.)	20.17	23.38
	Air-breathing fishes		
6	*Macrones gulio* (Ham.)	31.42	35.38
7	*Heteropneustes fossilis* (Bloch)	29.41	36.23
8	*Boleopthalmus dussumieri* (Cuv. & Val.)	30.40	37.60
9	*Osphronemus gourami* (Lacep)	32.17	36.96
10	*Ophiocephalus striatus* (Bloch)	32.87	40.00

N.B.:—The specimens of fishes collected for this piece of investigation comprise both fresh-water and marine teleosts. The water-breathing Tetrodon and the air-breathing Macrones and Boleopthalmus were collected from Mahim creek. The water-breathing marine Glyphidodon was collected from the sea-coast of Chowpatty, Bombay. All the fresh-water water-breathing fishes as well as the air-breathing Osphronemus and some specimens of Ophiocephalus were collected from the Bandra tank. Other specimens of Ophiocephalus were collected from the river Sabarmati at Ahmedabad, while the specimens of Heteropneustes were collected from a tank situated in the Aarey Milk Colony, Goregaon, Bombay.

Discussion

The haemoglobin concentration of blood based on iron estimation has been estimated by a number of workers including Hall and Gray (1929). The results obtained by Hall and Gray (1929) are as follows:—

	Name of the fish	The amount of iron estimated in mg. in 100 cc. of the blood	
		Minimum	Maximum
	Water-breathing fishes		
1	Rudder fish	17.3	25.0
2	Puffer fish	17.1	27.8
3	Toad fish	12.5	15.2
4	Bonito	37.0	52.9
5	Mackerel	34.3	51.0

Root (1931), Redfield (1933), Willmer (1934) and Black (1940) have estimated the oxygen-carrying capacity of the blood by determining oxygen present per 100 cc. of the blood. Some of the results obtained by them in certain teleostean fishes are as follows :—

	Name of the fish	Mean oxygen-carrying capacity cc. oxygen/100 cc. blood
	Water-breathing fishes	
1	Goose fish	5.10
2	Puffer fish	6.80
3	Toad fish	6.20
4	Paku (*Myleus setiger*)	10.78
5	Haimara (*Hoplias malabaricus*)	6.53
6	Common sucker (*Catostomus*)	10.60
7	Carp (*Cyprinus carpio*)	12.50
8	Cat fish (*Ameiurus nebulosus*)	13.30
9	Mackerel	15.80
	Air-breathing fishes	
10	Eel (*Anguilla japonica*)	12.90
11	Eel (*Electrophorus electricus*)	19.70
12	Hossa (*Hoplosternum littorale*)	18.14

The results obtained by Hall & Gray (1929), as seen from their investigations based on the estimation of iron, show that the two migratory fishes Bonito and Mackerel have a higher oxygen-loading tension of blood than the non-migrating water-breathing fishes. Their investigations show that the amount of iron present in the blood of non-migratory fishes varied from a minimum of 12.5 mg. in toad fish to a maximum of 27.8 mg. in the puffer fish. The corresponding figures for the two migratory fishes vary from a minimum of 34.3 mg. in Mackerel to a maximum of 52 9 mg. in Bonito.

The present investigations show that the haemoglobin concentration of the blood based on the estimation of iron is different for water-breathing and air-breathing fishes. For water-breathing teleosts, the amount of iron varies from a minimum of 15.13 mg. in *Labeo rohita* to a maximum of 27.52 mg. in *Catla catla*. The corresponding figures for air-breathing fishes vary from a minimum of 29.41 mg. in Heteropneustes to a maximum of 40.0 mg. in Ophiocephalus.

The investigations of Hall & Gray (1929), Redfield (1933), Willmer

(1934) and Black (1940) show that besides air-breathing fishes, the oxygen-loading tension is higher in certain other fishes also. These fishes include the well known migratory marine fishes Bonito and Mackerel, *Myleus setiger*, an active fish of the open river, a swampy acid-water inhabiting cat-fish, Ameiurus and sucker fish, Catostomus. A higher oxygen-carrying capacity of the blood of Bonito and Mackerel is perhaps an index of their physiological activity as stated by Hall and Gray (1929). The same seems to be the case with *Myleus setiger*. As regards Ameiurus, it seems to be a safeguard against the difficulties encountered by the fish during respiration, on account of the peculiar nature of its habitat known to contain acidic water as well as higher amount of carbon dioxide in its waters. Such conditions are known to affect the utilization of oxygen in a number of fishes (Fry, 1957). Can the higher amount in the blood of Catostomus which is a sucker fish be explained as correlated to its mode of life as an attached fish? It could be done if its respiratory movements are impeded on account of attachment. If that is not so, some other explanation has to be found for the higher oxygen-capacity of its blood.

The blood of a non-migratory water-breathing fish, *Cyprinus carpio* (Redfield, 1933 and Black, 1940) is also known to have a higher oxygen tension. Not much is known about this fish except it is a sluggish form. The authors have not given any explanation for the high value of the oxygen tension of its blood.

The air-breathing fishes studied during the present investigation include *Ophiocephalus striatus* and *Heteropneustes fossilis* which normally are not very active. The water-breathing fishes such as *Cirrhina mrigala*, *Labeo rohita* and *Catla catla* inhabiting similar waters are comparatively more active. So, a higher concentration of iron in the blood of the air-breathers cannot be explained on the basis of their activity, since they are inactive nor could it be explained on their adaptations to the peculiar conditions of their environments because normal water-breathers also inhabit the same environment. An explanation other than one based on activity or the nature of the environment, must therefore be found for the higher percentage of iron present in the blood of the air-breathers. The only reasonable explanation seems to be that this feature is in some way intimately related to the air-breathing habit.

It means therefore that the mutations which induced the growth of aerial respiratory organs were correlated with those inducing an increase

in the oxygen-fixing capacity of the blood. Such combination of traits in air-breathing fishes can be regarded as an example of the principle of functional correlation so common in evolution.

Summary and Conclusions

1. Estimation of iron in mg. per 100 c.c. of the blood in 5 water-breathing and 5 air-breathing teleosts has been carried out to determine its oxygen-carrying capacity.

2. The amount of iron has been found to vary from a minimum of 15.13 mg. to a maximum of 27.52 mg. in water breathers. The corresponding figures for air-breathers are 29.41 mg. and 40.00 mg. respectively. These results show that the loading tension of oxygen in the blood of air-breathers is higher than that in water-breathers.

3. It is suggested that the aquisition of the air-breathing habit is closely correlated with the oxygen-fixing capacity of the blood.

Acknowledgements

The author expresses his thanks to Prof. C. J. George, formerly of Wilson College, Bombay for suggesting the problem and also for the facilities afforded for conducting the investigations. Thanks are also due to Prof. J. C. George for his keen interest in the work.

References

1 Baldwin, E. 1949 Introduction to Comparative Biochemistry, Cambridge Univ. Press.

2 Black, E. C. 1940 The transport of the oxygen by the blood of fresh-water fish. *Biol. Bull.*, 79 (1), 215-229.

3 Dubale, M. S. 1957 Certain aspects of air-breathing in fishes with special reference to Indian teleosts. Thesis accepted by M. S. University Baroda, for Ph.D. degree.

4 Day, F. 1868 The Fauna of British India—Fishes, Vol. I and II. Taylor and Francis, London.

5 Erichson, J. 1947 The oxygen consumption of *Gasterosteus aculeatus* L. in toxic solutions. *J. Exp. Biol.*, 23.

6	Fry, F. E. J. and Black, E. C.	1939	The influence of carbon dioxide on the utilisation of oxygen by certain species of fish in Toronto region. *Amer. J. Physiol.*, 126, 497-498.
7	Fry, E. F. J.	1957	The acquatic respiration of fish from The Physiology of Fishes, Vol. I, 1-55. Academic Press Inc., New York.
8	Gause, G. F.	1942	The relation of adaptability to adaptation. *Quart. Rev. Biol.*, 17.
9	Hall, F. G. and Gray, I. E.	1929	Haemoglobin concentration of marine fishes. *J. Biol. Chem.*, 81, 589-594.
10	Hall, F. G., Gray, I. E. and Lepkovsky.	1926	The influence of asphyxiation on the blood conditions of the marine fishes. *J. Biol. Chem.*, 77, 549-554.
11	Hawk, Oser and Summerson.	1949	Practical Physiological Chemistry. J. and A. Churchill Ltd., London.
12	Heilbrunn, L. V.	1952	An Outline of General Physiology. Saunders, Philadelphia.
13	Kennedy, R. P.	1927	The quantitative determination of iron in tissues. *J. Biol. Chem.*, 74, 385-391.
14	Mitchel, P. H.	1938	General Physiology. McGraw-Hill Book Co., New York.
15	Mott, J. C.	1957	The Cardiovascular system—from The Physiology of Fishes, Vol. I, 82-108. Academic Press Inc., New York.
16	Parr, A. A.	1926	Adaptiogenese and Phyogenese-zor analyse der anapassunger scheinungen und ikre, enstechung. *Theo. Organ. Ent.*, 1, 1-60.
17	Prosser, C. L. *et al*	1950	Comparative Animal Physiology. Saunders, Philadelphia.

18 Redfield, C. A. 1933 Evolution of the respiratory function of the blood. *Quart. Rev. Biol.*, 8, 31-57.

19 Rogers, C. G. 1938 Text book of comparative Physiology. McGraw-Hill Book Co., New York.

20 Root, R. W. 1931 The Respiratory function of the blood of the marine fishes. *Biol. Bull.*, **61**. 427-456.

21 Willmer, E. N. 1934 Some observations on the respiration of certain tropical fresh-water fishes. *J. Exp. Biol.*, **11**. 283-306.

THE PIGEON BREAST MUSCLE LIPASE

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THE presence of esterases in muscle is known since long (Matlack and Tucker, 1940). Recently it was shown that the *pectoralis major* muscle of the pigeon and other highly active muscles of vertebrates and insects too contain a true lipase (George and Scaria, 1956, 1957; George, Susheela and Scaria, 1958a, George, Vallyathan and Scaria, 1958). The presence of this enzyme in these muscles was histochemically demonstrated using "Tween 80" as substrate. (George and Scaria, 1958a, 1958b; George, Susheela and Scaria, 1958a; George, Vallyathan and Scaria, 1958). Later it was decided by the authors to study the properties of the pigeon breast muscle lipase and incidentally those of the pancreatic lipase too, under identical conditions, with a view to comparing them. Since the properties of the pancreatic lipase revealed by our study were in many respects different from earlier accounts on the pancreatic lipase by other authors, it was decided to present them separately in another paper. Here we report only the properties of the pigeon breast muscle lipase.

Material and Methods

In our histochemical study of lipase in muscle and pancreas we observed that treatment of the tissues with acetone completely destroys the enzymic activity. It was also observed that the lipolytic activity of acetone powder prepared according to the usual methods was nil. Acetone probably destroys the enzyme. It was therefore thought desirable to prepare a powder without treatment with acetone. Reviewing the literature, it was found that an ether defatted dry powder of *Fusarium lini* Bolly was used as the enzyme material by Fiore and Nord (1950) for their studies on this mould lipase. This method was tried and found highly satisfactory. The powder was prepared in the following manner. Pigeons were decapitated and the *pectoralis major* muscle removed when all the blood was drained off. The muscle was then minced with a knife, spread into thin layers in large petri dishes and dried *in vacuo* in a desiccator over calcium chloride. The dehydrated muscle was then turned

into a fine powder in a mortar and sieved through fine silk to remove the tendon and other coarse particles. The powder was then treated with a large quantity of cold ether for 1 hr., during which much of the fat was removed. It was then filtered, washed with more ether and dried at room temperature till all the ether was completely removed. This powder can remain for a long time in the refrigerator without much loss of the enzymic activity. Aqueous extracts of this powder was used as the enzyme solution in the present series of experiments.

500 mg. of this powder was extracted in 5 ml. water in a small mortar in cold for 1 hr., centrifuged at about 2500 r.p.m. and the supernatant used as the enzyme solution. 1 ml. of this solution contains 13 to 14 mg. of protein. Protein was estimated colorimetrically according to the micro-Kjeldahl method (Hawk *et al* 1954). This protein does not contain –SH or –S–S– groups as revealed by the fact that the nitroprusside reaction for –SH and the lead-blackening test for sulphur (Hawk *et al*, 1954) are negative. The enzyme solution when treated with BAL (British-Anti-Lewisite, 2:3: dimercaptopropanol) forms a blue green precipitate which according to the descriptions of Webb and van Heyningen (1947) and Barron *et al* (1947) is a BAL-iron compound. A solution in 3N HCl of the ash of this precipitate was found to contain large quantities of iron.

Lipase activity was manometrically determined using the Warburg apparatus, in a bicarbonate-CO_2 buffer system of pH 7.4 at 37°C. The substrate used was a 4% (v/v) emulsion of tributyrin in 0.0148 M $NaHCO_3$, prepared by shaking with 1 or 2 drops of "Tween 80." in a small conical flask. Each reaction flask contained 1.5 ml. 0.025 M $NaHCO_3$, 0.5 ml. of the substance under test in concentrations to give the final concentrations as indicated below and 0.5 ml. enzyme in the main chamber and 0.5 ml. substrate in the side arm in a total volume of 3 ml. This gives a final concentration of 0.0148 M $NaHCO_3$ with a pH of 7.4 (Umbreit *et al*, 1951). The test solution was introduced into the reaction flask before the addition of the enzyme, except in cases where it is otherwise stated. The flasks and manometers were gassed for 3 min. with a mixture of 95% N_2 and 5% CO_2. After equilibration for 10 min. in the constant temperature water bath, the substrate was tipped in and allowed to equilibrate for another 3 min. This period is sufficient to ensure complete mixing of the contents of the flask. The readings were taken at regular intervals for 1 hr.

For each experiment a control was run in which 0.5 ml. distilled water was added in place of the solutions under test. Autohydrolysis was nil. The readings given are after correction for the thermobarometer. The control produced on the average 11 to 12 μmoles of CO_2 equivalent to the amount of butyric acid liberated,

Results

Effect of various Substances

Inorganic salts: (Figs. 1 and 2) $MgCl_2$ at low concentrations slightly activated the enzyme. NaCl, KCl, $CaCl_2$ and NH_4Cl were inhibitory.

Intermediate metabolites: (Fig. 3) At concentration of 0.0025 M, all the substances except citrate activated the enzyme to a limited extent. Highest activation was obtained in the case of ∝ ketoglutarate at a concentration of 0.01 M.

Amino acids: (Fig. 4) Both the amino acids tested *viz.* histidine and L-methionine were inhibitory. At a concentration of 0.01 M the inhibition by methionine was only 4% and by histidine about 25%.

Adenosinetriphosphate (ATP): (Fig. 5) Slightly activated the muscle lipase. There was no inhibition upto a concentration of 10^{-3} M.

Heparin: (Fig. 6) Inhibited the enzyme about 12% at a concentration of 40 μ g/ml.

Urethane: (Fig. 7) Slightly activated the enzyme at a concentration of 0.2%. At higher concentrations the enzyme was inhibited, the inhibition being about 12% at a concentration of 1%.

Sodium taurocholate: (Fig. 8) Was found to inhibit the enzyme to the extent of about 50% at a concentration of 0.01 M.

Metal chelating agents: 8-hydroxyquinoline is known to bind many metals in biological materials (Albert and Gledhill, 1947). The solubility of this substance in water is extremely low. A saturated aqueous solution was prepared (31°C) and 1.5 ml. of this solution was added to 1.5 ml. of the enzyme and incubated for 30 min. at 37°C. A control was prepared by similarly incubating 1.5 ml. of the enzyme with 1.5 ml. water. 1 ml. each of these pre incubated enzymes was added to each flask and the enzyme was found to be inhibited about 4.5% by this reagent,

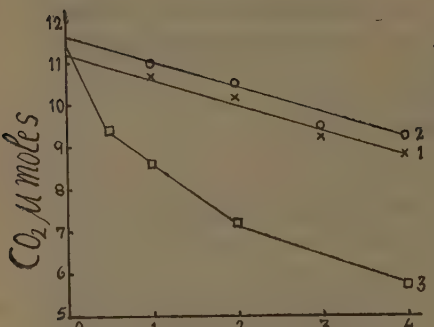


Fig. 1

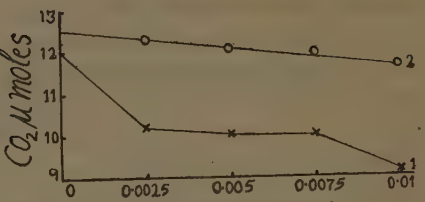


Fig. 4

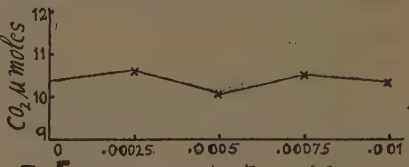


Fig. 5

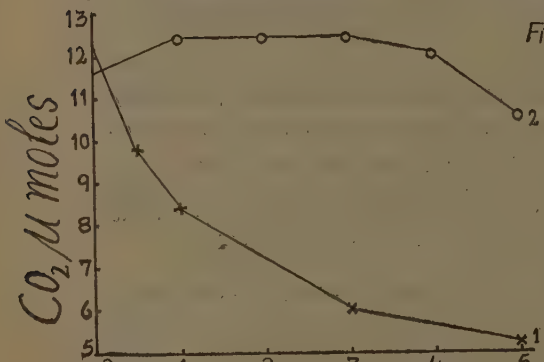


Fig. 2

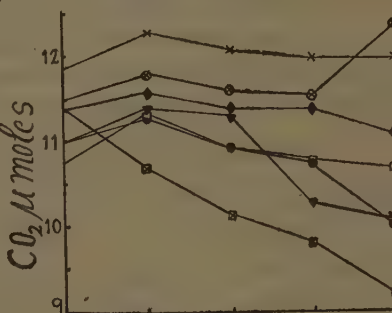


Fig. 3

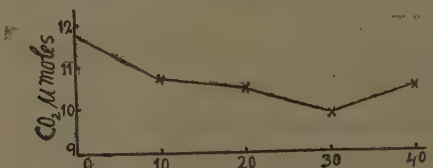


Fig. 6

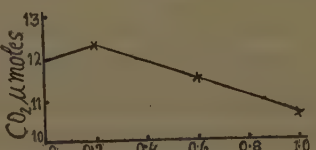
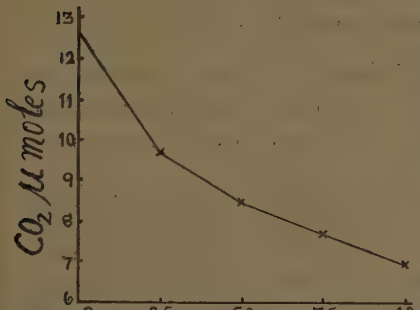
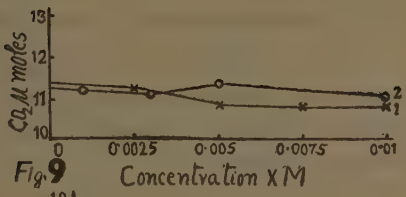


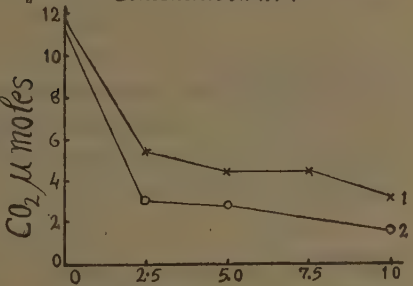
Fig. 7

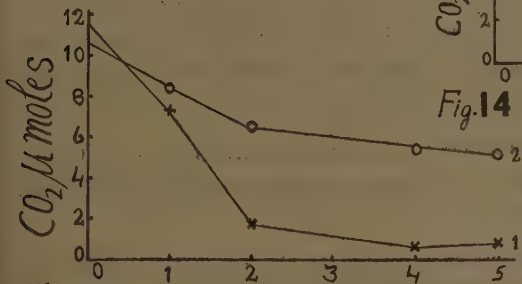
EXPLANATION OF FIGURES

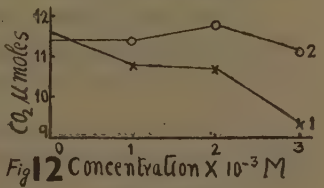
Effect of various substances on the pigeon breast muscle lipase. Fig. 1. 1, NaCl; 2, KCl; 3, N
Fig. 2.1, $CaCl_2$; 2, $MgCl_2$. Fig. 3. 1, Pyruvate; 2, Succinate; 3, Fumarate; 4, Malate; 5, Lactate; 6, c
glutarate; 7, Citrate. Fig. 4. 1, Histidine; 2, L-methionine. Fig. 5. ATP. Fig. 6. Heparin. Fig. 7. Ure

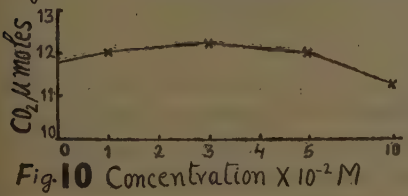


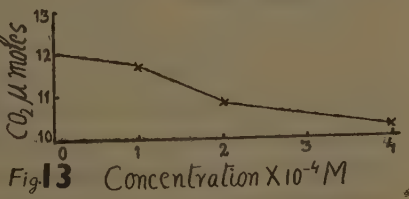












Effect of various substances on the pigeon breast muscle lipase. Fig. 8. Sodium taurocholate. Fig. 9. 1, Iodoacetate; 2, Potassium ferricyanide. Fig. 10. Thioglycollate. Figs. 11 & 12. 1, Glutathione 2, Cysteine. Fig. 13. BAL. Fig. 14. 1, PCMB; 2, $HgCl_2$.

Thiol reagents. Alkylating agent: Iodoacetate—The enzyme was not affected much by this reagent at concentrations upto 10^{-2} M (Fig. 9).

Oxidizing agent: Potassium ferricyanide at concentrations upto 10^{-2} M was without any action on this enzyme (Fig. 9).

Reducing agents: Monothiols—Thioglycollate slightly activated the enzyme at concentrations below 0 05 M. At higher concentrations it inhibited the enzyme. Only 4% inhibition was noticed at a concentration of 0.1 M (Fig. 10).

Glutathione and cysteine: Both these substances were inhibitory. Glutathione was more effective as an inhibitor. At a concentration of 0.05 M, the enzyme was inhibited 90% by glutathione and about 50% by cysteine (Fig. 11). At lower concentrations, *viz.* 0.001 to 0.003 M, glutathione still inhibited the enzyme, but cysteine activated it slightly at a concentration of 0.003 M (Fig. 12).

Dithiol: At all concentrations BAL inhibited the enzyme. The inhibition was directly related to the concentration of the substance added (Fig. 13).

Mercaptide forming substances: *p*-Chloromercuribenzoate (PCMB) at all concentrations was inhibitory. At a concentration of 0.0025 M, the inhibition was about 75% (Fig. 14).

Mercuric chloride: At the same concentrations as that of PCMB, $HgCl_2$ inhibited the enzyme more effectively (Fig. 14). Even at very low concentrations of $HgCl_2$, the enzyme was considerably inhibited.

Effect of pre-incubating the enzyme first with $HgCl_2$ and then with BAL. To 1.5 ml. of the enzyme solution was added 0.5 ml. 2.4×10^{-4} M $HgCl_2$ and incubated for 15 min. at 37°C. A control was also similarly incubated side by side with 0.5 ml. H_2O added to 1.5 ml. of the enzyme (tube 1). To 1 ml. of the former was added 1 ml. H_2O (tube 2) and to the remaining, 1 ml. 6×10^{-4} M BAL. To tube 1 was added 2 ml. H_2O so as to make the concentration of the enzyme the same in all the three tubes. All the tubes were again incubated for 15 min. and 1 ml. each was added to the Warburg flasks in duplicate and the activity of the enzyme determined as usual. The results are given in Table 1. Flask 2 had a final concentration of 10^{-5} M $HgCl_2$ and flask 3 contained 10^{-5} M $HgCl_2$ and 10^{-4} M BAL.

Activity, µl CO_2 liberated		
Control, without addition (1)	Enzyme + $HgCl_2$, 10^{-5} M (2)	Enzyme + $HgCl_2$, 10^{-5} M + BAL, 10^{-4} M (3)
285	271	246

Table 1. Effect of pre-incubating pigeon breast muscle lipase with $HgCl_2$ and BAL.

It can be seen that the enzyme is slightly inactivated by $HgCl_2$. The addition of BAL brings about a further inhibition of the enzyme by 9%.

In another set of experiments in which the concentration of $HgCl_2$ and BAL was ten times that in the previous experiments the following results were obtained (Table 2).

Activity, µl CO_2 liberated		
Control, without addition (1)	Enzyme + $HgCl_2$, 10^{-4} M (2)	Enzyme + $HgCl_2$, 10^{-4} M + BAL, 10^{-3}M (3)
257	200	250

Table 2. Effect of pre-incubating pigeon breast muscle lipase with $HgCl_2$ and BAL.

At this concentration the muscle lipase was inhibited 22% by $HgCl_2$ and the inhibition was almost completely reversed by BAL.

Effect of preincubating the enzyme first with BAL and then with $HgCl_2$. 1.5 ml. of the extract was incubated for 15 min. with 0.5 ml. 6×10^{-3} M BAL at 37°C. A similarly incubated enzyme to which 0.5 ml. H_2O was added was used ascontrol (tube 1). To 1 ml. of the former was added 1 ml. H_2O (tube 2) and to the remaining, 0.6 ml. 2.4×10^{-4} M $HgCl_2$ and 0.4 ml. H_2O (tube 3). 2 ml. H_2O was added to tube 1 and the tubes incubated for a further period of 15 min. 1 ml. each of these preparations was added to the flasks and the activity noted. The final concentration of BAL and $HgCl_2$ in the

flasks was 2.5×10^{-4} M and 2.4×10^{-4} M respectively. The results are given in table 3.

Activity, µl CO_2 liberated		
Control, without addition (1)	Enzyme + BAL, 2.5×10^{-4} M (2)	Enzyme + BAL, 2.5×10^{-4} M + $HgCl_2$, 2.4×10^{-4} M (3)
223	175	170

Table 3. Effect of pre-incubating pigeon breast muscle lipase with BAL and $HgCl_2$.

22% of the activity of the enzyme was inhibited by BAL. This inhibition was not reversed by $HgCl_2$.

Discussion

The studies on enzymes hydrolysing fats and esters have recently been extensively reviewed by Ammon and Jaarma (1950). It is known that lipases from different sources differ from each other with regard to their solubility, specificity and kinetic properties. Even though there is a certain degree of overlapping of substrate specificity between the non-specific esterases and lipase, it is generally agreed that the enzyme hydrolysing tributyrin is a true lipase (Desnuelle, 1951). From the results presented in this paper, it is clear that the muscle lipase hydrolyses tributyrin rapidly. In a titrimetric system also this enzyme readily hydrolyses tributyrin, but the hydrolysis of olive oil is very slow (George and Scaria, 1956). The rate of hydrolysis of olive oil by the pigeon pancreatic lipase is likewise very slow compared to the action on tributyrin. However, in a manometric system olive oil is not hydrolysed by any of these lipases, an observation also made by Martin and his colleagues (Martin and Peers, 1953) using oat lipase. The non-hydrolysis of olive oil in such a system, according to these authors, is due to the predominance of water in the system.

The purpose of the present study was to compare the properties of the muscle enzyme with those of the pancreatic lipase. The studies on the latter enzyme is reported elsewhere (George and Scaria, 1959). It was suggested that each of these enzymes is adapted for maximal activity in the physiological environment in which it acts, on the grounds that the

pancreatic lipase is activated by sodium taurocholate and inhibited by intermediary metabolites. The action of muscle lipase also supports this assumption. This enzyme is inhibited by sodium taurocholate and is activated by intermediate metabolites. It should be remembered that the pancreatic lipase acts in an environment rich in bile salts which are promoters of digestion. The muscle lipase on the other hand is intracellular and the metabolic pool constitutes its physiological environment. The exact mode of activation of pancreatic lipase by bile salts is not understood. The present study shows that the activation is not due to the emulsification of the substrate by reducing its surface tension, as is commonly believed. The fact that ATP does not inhibit the muscle lipase up to a concentration of 10^{-3} M further supports this assumption. It should be noted here that at this same concentration the pancreatic lipase is inhibited about 56% by ATP (George and Scaria, 1959) Heparin inhibits the muscle enzyme as well as the pancreatic enzyme. The enzyme does not require any cation for its activity. The possibility that the enzyme requires cations for its activity and the required cations in sufficient amounts may be present in the muscle enzyme preparation is not excluded. At any rate added cations have only an inhibitory effect except in the case of Mg, which shows a slight activation at low concentrations.

The negative results obtained with the nitroprusside reaction and the lead-blackening test indicate that the enzyme does not contain either –SH groups or –S–S– groups in its protein. The pancreatic enzyme also gave the same results. In this context it is interesting to note the behaviour of this enzyme in the presence of sulphydryl reagents. According to Dickens (1933) iodoacetate reacts by substitution of the hydrogen of the sulphydryl by the carboxymethyl group. Michaelis and Schubert (1934) however have noted that acid halides combine with amino groups. Both potassium ferricyanide and iodoacetate inhibit the pancreatic lipase. The inhibition by the former is not due to the oxidation of the –SH group and the inhibition by the latter is explained as due to combination with reactive NH_2 groups (George and Scaria, 1959). These chemicals, however, do not appreciably inhibit the muscle lipase at concentrations used for the pancreatic lipase. This may be due to the fact that the protein content of the muscle lipase preparation is about 20 times that of the pancreatic lipase preparation and there is competition between the enzyme protein and the non-enzymic protein for the inhibitor.

An alternative suggestion is that it may be due to interaction with other substances present in the enzyme solution.

The muscle lipase apparently differs from the pancreatic lipase in that the latter is activated by $HgCl_2$ at very low concentrations, while the former is not. This may not be a real difference, if, the activation by $HgCl_2$ is due to the binding of some inhibitory substance present in the enzyme. PCMB, just like $HgCl_2$, inhibits the enzyme at all concentrations. This inhibition may be due to the binding of reactive NH_2 groups of the protein because there are no—SH groups in the enzyme with which these substances can form mercaptides. It is known that the mercaptide forming substances can also combine with the amino groups of proteins (Barron, 1951). It may be noted that Little and Caldwell (1943) have shown that freely reactive NH_2 groups are required for the activity of pancreatic lipase.

It has been suggested that the pigeon pancreatic lipase may be a metallo-protein or an enzyme requiring metal for its activity and the reactivity of the enzyme with reducing substances such as monothiols and dithiols may be due to reaction with the metal part required for activity and not with any -SH or -S-S- groups. Thiols combine with metals forming mercaptides of varying degrees of reversibility. The inhibition of metal containing enzymes by BAL is believed to be due to this reaction. (Barron *et al*, 1947).

$$Me + 2\,RSH \rightleftarrows Me(RS)_2 + 2H^+$$

The muscle lipase preparation contains a large amount of iron. This iron might be mostly from the myoglobin and other soluble substances present in the muscle. However, when the metal was precipitated by BAL, the enzyme was inactivated, showing that some metal is essential for its activity. The insoluble blue green precipitate formed with BAL is a BAL-iron compound (Barron *et al*, 1947; Webb and van Heyningen, 1947). The essential metal may also be one like Mg. which can form soluble metal complexes with BAL. The reactivation by BAL of the enzyme, inhibited by $HgCl_2$ may be due to the removal of the inhibitory effect by combining with this metal for which BAL has a greater affinity. However, unlike the pancreatic lipase, the inhibition of the enzyme by BAL is not reversed by the addition of $HgCl_2$. The inhibition of the muscle lipase by 8-hydroxyquinoline though to a lesser degree also suggests that it may be a metal requiring enzyme. The failure of the chemical in inhibiting the

enzyme to a greater extent may be explained as due to the extremely low concentration of the chemical used and the very high concentration of metals present in the enzyme solution. Higher concentrations of this reagent could not be tested because of its very low solubility in water. Monothiols like cysteine can also form complexes with metal ions (Warburg and Sakuma, 1923). The inhibition of muscle lipase by BAL can be therefore explained as due to formation of complexes with metal ions required for its activity. The slight activation of the enzyme by cysteine and thioglycollate at low concentrations may be due to the binding of inhibitory metal ions. Whereas this enzyme is activated by low concentrations of cysteine, the pancreatic lipase is activated by low concentrations of glutathione. The ability of these substances to combine with different metals varies considerably (Barron *et al*, 1947; Webb and van Heyningen, 1947).

In conclusion it may be said that the muscle lipase in all essential features of its activity resembles the pancreatic lipase. Both appear to be enzymes requiring metal ions and reactive NH_2 groups for activity and are devoid of reactive -SH or -S-S- groups. No requirement of added cations could be demonstrated. Heparin is inhibitory to both the enzymes. The difference in the reactivity of these enzymes in the presence of various substances is due to interference with inhibitory or activating substances present in the crude undialysed enzyme preparations.

Summary

Certain biochemical properties of the pigeon breast muscle lipase are studied and compared with those of the pigeon pancreatic lipase. Added cations are not required for the activity of this lipase. This enzyme appears to be devoid of either -SH or -S-S- groups in its molecule and one requiring metal ions and reactive NH_2 groups for its activity. Comparative tests with pancreatic lipase have shown that there are certain apparent differences between the two lipases. Despite these differences pointed out, there are not sufficient grounds to regard this enzyme to be different from the pancreatic lipase.

References

Albert, A. and Gledhill, W. S. 1947 *Biochem. J.*, 41, 74.

Ammon, R. and Jaarma, M. 1950 The Enzymes. Ed. Sumner and Myrback, Vol. 1, Academic Press, New York.

Barron, E. S. G. *et al*	1947	*Biochem. J.*, **41**, 62.
Barron, E. S. G. *et al*	1947	*Ibid.*, **41**, 78.
Barron, E. S. G.	1951	Advances in Enzymology. Ed. Nord, Vol. 11, Inter-science, New York.
Desnuelle, P.	1951	As cited by Martin and Peers.
Dickens, F.	1933	*Biochem. J.*, **27**, 1141.
Fiore, J. V. and Nord, F. F.	1950	*Archiv. Biochem.*, **26**, 382.
George, J. C. and Scaria, K. S.	1956	*J. Anim. Morph. Physiol.*, **3**, 91.
George, J. C. and Scaria, K. S.	1957	*Ibid.*, **4**, 107.
George, J. C. and Scaria, K. S.	1958b	*Ibid.*, **5**, 43.
George, J. C. and Scaria, K. S.	1958a	*Nature*, **181**, 783.
George, J. C. and Scaria, K. S.	1959	Under preparation for publication.
George, J. C., Susheela A. K. and Scaria, K. S.	1958a	*Naturwissenschaften*, **45**, 93.
George, J. C., Vallyathan, N. V. and Scaria, K. S.	1958	*Experientia*, **14**, 250.
Hawk *et al*	1954	Practical Physiological Chemistry. Mc Grow-Hill, New York.
Little, J. C. and Caldwell, M.L.	1943	*J. Biol. Chem.* **147**, 229.
Martin, H. F. and Peers, F. G.	1953	*Biochem. J.*, **55**, 523.
Matlack, M. B. and Tucker, I. W.	1940	*J. Biol. Chem.*, **132**, 663.
Michaelis, L. and Schubert, M. P.	1934	*Ibid.*, **106**, 331.
Umbreit, *et al*	1951	Manometric Techniques and Tissue Metabolism. Burgess Publishing Co., Minneapolis, Minn.
Warburg, O. and Sakuma	1947	As cited by Barron *et al*, *Biochem. J.*, **41**, 62.
Webb, E. C. and van Heyningen, R.	1947	*Biochem. J.*, **41**, 74.

RESULTS OF FRACTIONAL GASTRIC ANALYSIS EXAMINATION IN GASTRIC DISORDERS
(Part II)

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RESULTS of gastric examination by fractional test meal method were reported from this laboratory (Pai, 1956 and 1957). In this paper results of further analysis of the examination of gastric secretory function by fractional method are set forth.

Materials and Methods

Forty-six cases, admitted for complaints of gastric troubles, out of whom forty were males and six females, of age varying between 16 and 66 years, coming from this part of the country, namely, Gujarat, have been studied for their gastric secretory function by the technique of fractional Test meal, with the alcohol (Bloomfield and Keefer (1927), and/or histamine Test meal, (Bloomfield and Polland (1929)), according to the methods which were standardized and reported in the earlier communications (Pai, 1956 and 1957).

Results and discussion

The following criteria were given by Napier and Das Gupta (1935) and Napierchaudhari and Raichaudhari (1938) for classifying acid curves based on the highest free acidity obtained per 100 cc. of gastric juice :—

(1)	Achlorhydria	No free hydrochloric acid.
(2)	Hypochlorhydria	Free hydrochloric acid. 1 to 10 cc. N/10 acid %.
(3)	Isochlorhydria	Free hydrochloric acid— 11 to 65 cc. N/10 acid %.
(4)	Hyperchlorhydria	Free hydrochloric acid— more than 65 cc. N/10 acid %.

The ranges of the acidity readings in the above four groups of the forty-six cases with the mean values and the standard error calculated have been shown in Table I. The gastric samples aspirated were quali-

tatively analysed for the presence or otherwise of starch, mucus, blood, bile and lactic acid according to the method described in the earlier communication (Pai, 1957). In Table II, are shown the results of the percentage incidence of the positive tests obtained for the above abnormal constituents in the gastric samples. In the same table are also shown the results of the percentage incidence of the cases confirmed by histamine Test meal done on the second occasion, after the alcohol Test meal had been done first. The above results have been shown separately for each of the four groups classified as described above (vide Table II).

Achlorhydria :

Out of nine cases of achlorhydria as determined by alcohol meal test, five were confirmed as cases of true achlorhydria, whereas the remaining four were found to be pseudoachlorhydric, after the histamine meal test. Histamine meal examination thus becomes an important test for determining the secretory power of the stomach under standard but unphysiological conditions. One patient, showing pseudoachlorhydric response, with highest free acidity of 7 cc. N/10 acid % after histamine meal test, was found to be suffering from ankylostomiasis as well. Bile was found to be present in the gastric samples of 11% of the total number of cases, and mucus in 33%, whereas none of the cases showed the presence of starch, lactic acid and blood in the gastric samples.

Hypochlorhydria :

In seven cases with hypochlorhydric response, the range of acidity was from 2.0 to 5.5 cc. with the mean of 4.3 ± 0.59 cc. N/10 acid %. The condition of ankylostomiasis was associated in five out of the seven cases. Thus the incidence of the occurrence of ankylostomiasis, which was 70% in this group, was the highest amongst all the four groups studied. Thus ankylostomiasis, is associated with a very low acidity of the gastric juice. This is in conformity with the observation made earlier (Pai, 1957). Bile was present in the gastric samples in 14%, mucus in 56% and starch in 14% of the cases. None of the cases showed the presence of either blood or lactic acid in the gastric samples. Histamine meal test confirmed the hypochlorhydria in 14% of the cases.

Isochlorhydria :—

Eighteen cases gave the isochlorhydric response. In only 6% of the cases, ankylostomiasis was associated. The range of acidity was from 14.0 to 40.0 with the average of 25.4 ± 2.05 cc. of N/10 acid %. Histamine

TABLE I

Results of fractional gastric analysis, showing the range of highest free acidity, with the average thereof and the standard error

Type of response after the fractional gastric analysis examination	Total No. of subjects	*Highest free hydrochloric acid in cc. N/10 acid %*			
		Maximum	Minimum	Mean	Standard error
(1) Achlorhydria	9	0	0	0	—
(2) Hypochlorhydria	7	5.5	2.0	4.3	± 0.59
(3) Isochlorhydria	18	40.0	14.0	25.4	± 2.05
(4) Hyperchlorhydria	12	100.0	72.0	82.3	± 2.80

TABLE II

Results of the gastric analysis by fractional method, showing the percentage incidence of the qualitatively detected substances

Condition	Range of the highest free acidity in cc. N/10 acid %	*Percentage Incidence of the presence of the qualitatively detected substances*							
		Confirmed after histamine meal test	Blood	Bile	Mucus	Starch	Lactic acid	Conditions of ankylostomiasis associated with in %age of cases
Achlorhydria	Absence of free HCl	In all:— Pseudo-achlorhydria in 44%. True achlorhydria in 56%	nil	11	33	nil	nil	11
Hypochlorhydria	2.0 to 5.5	14	nil	14	56	14	nil	70
Isochlorhydria	14.0 to 40.0	12	6 (may be due to injury while introducing the tube)	30	30	nil	6	6
Hyperchlorhydria	72 to 100.0	16	40% (from amongst peptic ulcer cases whose incidence was 50%)	24	8	8	nil	16

meal test was done in 12 % of the cases, for confirmation. Bile and mucus were present in gastric samples in 30% of the cases, starch in none and lactic acid and blood in 6%. The presence of blood might have been due to injury caused while introducing the tube.

Hyperchlorhydria :

Twelve cases out of the total of 46 cases gave hyperchlorhydric response, with the range of acidity from 72.0 to 100.0, having the mean value of 82.3 $\pm$ 2.80 cc. of N/10 acid %. Ankylostomiasis was associated in 16% of the cases, whereas the incidence of peptic ulcer was 50%. Out of these 50% of the cases, in 40% was found the presence of blood in their gastric samples. Bile was present in the samples in 24% of all the cases, starch and mucus in 8%, whereas none showed the presence of lactic acid in their gastric samples. Histamine Test-meal was done in 16% of the cases.

Summary and conclusions

(1) Forty-six cases, admitted for complaints of gastric troubles, out of whom forty were males and six were females, of age varying between 16 and 66 years, coming from this part of the country, namely, Gujarat, were studied for their gastric secretory function by method of fractional gastric analysis with the alcohol and/or histamine Test meal. The gastric samples were analysed quantitatively for the acidity content and qualitatively for the presence or otherwise of blood, starch, mucus, lactic acid and bile.

(2) Out of the total of 46 cases, 9 gave achlorhydric response, 7 gave hypochlorhydric, 18 gave isochlorhydric and 12 hyperchlorhydric responses.

(3) The condition of ankylostomiasis was associated in 70% of the cases belonging to hypochlorhydric response, which was the highest incidence in this group when compared to the other groups. Thus an kylostomiasis is associated with a very low acidity of the gastric juice. This finding is in conformity with the similar finding already reported by the author.

(4) Histamine test meal confirmed true achlorhydria in 56% of the cases of the achlorhydric group. Thus histamine meal examination becomes an important test for determining the secretory power of the stomach under standard but unphysiological conditions.

(5) From amongst the cases of the hyperchlorhydric response, fifty per cent cases showed the incidence of peptic ulcer. Blood was found to be present in the gastric samples in forty per cent out of these peptic ulcer cases.

(6) The percentage incidence of the presence of blood, bile, mucus, starch and lactic acid in the gastric samples in the cases of the above four groups separately has been shown in a tabular form. Data has been statistically analysed to evaluate the results.

(7) The results obtained above have been discussed in the paper and their significance has been emphasized.

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References

Bloomfield, A. L. and Keefer, I. 1927 *Jour. Amer. Med. Assoc.* **88**, 168.

Bloomfield, A. L. and Polland, W. S. 1929 *Jour. Clin. Invest.* **7**, 45.

Napier, L. E. and Das Gupta, C. R. 1935 *Ind. Jour. Med. Res.* **23**, 455.

Napier, L. E.; Chaudhari, R. N. and Raichaudhari, M. N. 1938 *Ind. Med. Gaz.* **73**, 65.

Pai, M. L. 1956 *Jour. Anim. Morph. Physiol.* **3**, 41.

Pai, M. L. 1956 *Ibid* **4**, 54.

which published (only recognised abbreviations of journals, underline) Volume number (double underline) and page number. *e.g.*

Haines, R. W. 1934 The homologies of the flexor and adductor muscle of the thigh. *J. Morph.*, **56**, 21.

Titles of papers may be omitted altogether. Books should be listed as follows: Author's last name followed by initials, year of publication, title, publishers and place of publication. *e.g.*

Heilbrunn, L. V. 1952 An Outline of General Physiology. W. B. Saunders Company, Philadelphia.

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